





1652 \$13 PATENT \$3 \$13 \$13

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Wezel et al.

Serial No.: 09/749,185

Filed: December 26, 2000

For: REDUCING BRANCHING AND ENHANCING FRAGMENTATION IN CULTURING FILAMENTOUS MICROORGANISMS

.......

Confirmation No.: 6157

Examiner: M. Rao

Group Art Unit: 1652

Attorney Docket No.: 2183-4666US

CERTIFICATE OF MAILING

I hereby certify that this correspondence along with any attachments referred to or identified as being attached or enclosed is being deposited with the United States Postal Service as First Class Mail on the date of deposit shown below with sufficient postage and in an envelope addressed to the Commissioner for Patents, Washington, D.C. 20231.

April 16, 2003

Signa

Betty Vowles_

Name (Type/Print)

APP CEIVEI

APP 2 4 2003

APP 1800/2900

COMMUNICATION

Commissioner for Patents Washington, D.C. 20231

Sir:

Enclosed is a Priority Document 98202148.7 filed 26 June 1998 for the above-referenced application.

Respectfully submitted

Allen C. Turner

Registration No. 33,041 Attorney for Applicant(s)

TRASKBRITT, P.C.

P.O. Box 2550

Salt Lake City, Utah 84110-2550

Telephone: 801-532-1922

Date: April 15, 2003

ACT/bv

Document in ProLaw



Eur päisches **Patentamt**

Eur pean **Patent Office** Office eur péen des brevets



Bescheinigung

Certificate

Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr.

Patent application No. Demande de brevet n°

98202148.7

Der Präsident des Europäischen Patentamts; Im Auftrag

For the President of the European Patent Office Le Président de l'Office européen des brevets p.o.

R C van Dijk

DEN HAAG, DEN THE HAGUE, LA HAYE, LE

24/03/03



Europäisches Patentamt **European Patent Office**

Office européen des brevets

Blatt 2 der Bescheinigung Sheet 2 of the certificate Page 2 de l'attestation

Anmeldung Nr.: Application no.: Demande n*:

98202148.7

Anmeldetag: Date of filing: Date de dépôt:

26/06/98

Anmelder: Applicant(s): Demandeur(s):

Rijksuniversiteit te Leiden

2312 AV Leiden

NETHERLANDS

Bezeichnung der Erfindung: Title of the invention: Titre de l'invention:

Reducing branching and enhancing fragmentation in culturing filamentous microorganisms

In Anspruch genommene Prioriät(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

Staat:

Tag:

Aktenzeicher

State: Pays: Date:

File no. Numéro de dépôt:

Internationale Patentklassifikation: International Patent classification: Classification internationale des brevets: C12N15/31, C12N5/10, C07K14/36

Am Anmeldetag benannte Vertragstaaten: Contracting states designated at date of filing: Etats contractants désignés lors du depôt;

AT/BE/CH/CY/DE/DK/ES/FI/FR/GB/GR/IE/IT/LI/LU/MC/NL/PT/SE

Bemerkungen: Remarks: Remarques: Title: Reducing branching and enhancing fragmentation in culturing filamentous microorganisms

10

15

20

The invention relates to industrial microbiology, particular to fermentation technology and especially fermentation methods for filamentous microorganisms, in particular filamentous bacteria such as actinomycetes. The invention was made in a research program into mechanisms of growth of streptomycetes.

Gram-positive, aerobic, Streptomycetes . are filamentous soil bacteria, which belong to the order of actinomycetales. In an early stage of Streptomyces growth on a solid medium, spores germinate, and subsequently develop into a vegetative mycelium of branching hyphae (Chater and Losick, 1996). After environmental signals such as nutrient depletion, aseptate aerial hyphae are formed, growing on the vegetative hyphae, the latter being used as a substrate. Eventually, the aerial hyphae form uninucleoid cells that develop into hydrophobic spores, which are budded off from the tips of the hyphae. One of the striking features of of · of the order streptomycetes and other members actinomycetales is their ability to produce a wide variety of secondary metabolites, including many antibiotics, which are produced in temporal relation to the onset of morphological differentiation in surface-grown cultures (Chater, Miyadoh, 1993). The molecular processes regulating the events that lead to differentiation of Streptomyces are presently only superficially understood, although new and interesting 25 insights into the genetics of streptomycetes have come to light (reviewed in Champness and Chater, 1993; Chater, 1993).

Most streptomycetes only sporulate on solid media, while growth in liquid cultures is restricted to 30 formation of vegetative mycelium. This typically develops into intricate networks of hyphae, among others resulting in pellet formation, with only the most outwardly oriented sections showing high physiological activity, resulting in low yield of the desired product per unit of biomass. Furthermore, because of their filamentous morphology, high density fermentations of biotechnologically interesting streptomycetes often are highly viscous, resulting in a low biomass accumulation due to for instance aeration and mixing problems. From this perspective it is desirable that fragmentation of the mycelium in submerged cultures is stimulated, that branching of the mycelium is reduced and that in general the viscosity of the culture is reduced.

10

15

20

25

Cell division in all bacteria analysed so far involves the tubulin-like GTP-binding protein FtsZ, which polymerises into a ring at the prospected site of the septum, presumably forming the physical scaffold for the assembly of the cell division apparatus (reviewed in Lutkenhaus and Addinall, 1997). In Escherichia coli and Bacillus species many factors have been identified that are involved in cell division, but little is known about this process in actinomycetes. Here septum formation does not lead to actual cell division, and while in most bacteria ftsZ is essential, the gene has been shown to be dispensable for mycelial growth in Streptomyces coelicolor (McCormick et al., 1994).

In contrast to most actinomycetes, Streptomyces griseus shows the ability to sporulate in submerged cultures over a short time period, when grown in defined minimal media (Kendrick and Ensign, 1983; Ensign, 1988). Kawamoto and Ensign (1995a,b) identified a mutation in the gene ssgA that relieved repression of sporulation in rich media. SsgA encodes an acidic protein with a molecular mass of approximately 16 kDa that displays no significant homology to any known protein in the database. Furthermore, overexpression of ssgA resulted in fragmented growth and suppression of sporulation in submerged cultures of S. griseus. Western blot analysis with polyclonal antibodies

raised against SsgA revealed that expression of SsgA directly correlates to the onset of submerged sporulation, with the protein appearing shortly before spore formation (Kawamoto et al., 1997). Importantly, although sporulation and production of the antibiotic streptomycin are apparently linked in S. griseus, no suppression of streptomycin production was observed. Apparently, regulation of sporulation and antibiotic biosynthesis occur via separate pathways.

The present inventors have shown that the activity of SsgA is not limited to the organism in which it is found. The can advantageously be transferred thereby allowing more fragmented growth and/or organisms, reduced branching and/or reduced viscosity of the culture of many filamentous microorganisms, in particular actinomycetes and Steptomycetes. Ιt is particularly surprising organisms that do not even harbour an ssgA-like gene themselves still respond to the presence of the product of such a gene. Thus we demonstrate that introduction of ssqA into various bacteria, in particular actinomycetes that do not harbour an endogenous ssgA, results in suppressed branching and enhanced fragmentation of the mycelium in liquid culture, resulting in significantly lower viscosity of culture broths. In addition to autonomously replicating plasmids containing constitutively expressed ssgA, we devised a system that allows easy integration of the gene in the chromosome, with the advantage of high stability.

Thus the invention now provides a method for producing a filamentous bacterium showing reduced branching during growth, particularly growth in a liquid medium, comprising providing such a bacterium with the capability of having or expressing heterologous SsgA-activity, which activity in Streptomyces Griseus is encoded by an ssgA gene having the sequence.

25

30

1 ATGCGCGAGTCGGTTCAAGCAGAGGTCATGATGAGCTTCCTCGTCTCCGA

51 GGAGCTCTCGTTCCGTATTCCGGTGGAGCTCCGATACGAGGTCGGCGATC

5

- 101 CGTATGCCATCCGGATGACGTTCCACCTTCCCGGCGATGCCCCTGTGACC
- 151 TGGGCGTTCGGCCGCGAGCTGCTGGACGGGCTCAACAGCCCGAGCGG
- 10 201 CGACGGCGATGTGCACATCGGCCCGACCGAGCCCGAGGGCCTCGGAGATG

 - 301 ACGGCACCGCTGGTGGCGTTCCTCGACCGGACGGACAAGCTCGTGCCGCT

15

20

25

30

351 CGGCCAGGAGCACACGCTGGGTGACTTCGACGGCAACCTGGAGGACGCAC

401 TGGGCCGCATCCTCGCCGAGGAGCAGAACGCCGGCTGA

As explained above the presence of additional SsgAactivity, in particular heterologous Scgn-activity (meaning activity not in a form as present in the microorganism in nature) leads to enhanced fragmentation, reduced branching and thus reduced viscosity. The activity may be provided in any suitable manner, but it is preferred that the activity is provided by transfecting or transforming said filamentous bactrium with additional genetic information encoding said activity. Examples of such methods are presented hereinbelow, but the art of genetic engineering of bacteria is so well advanced that persons skilled in the art will be able to come up with numerous methods and variations thereof to provide a intended filamentous bacterium with a gene encoding SsgA-like activity. SsqA-like activity is functionally defined as the ability to enhance fragmentation and/or reduce branching in (typically) submerged cultures of filamentous microorganisms,

in particular bacteria, more specifically actinomycetes. The activity of other ssgA-like genes or fragments of ssgA genes or derivatives of ssgA genes which are within the invention must be functionally the same, but that does not mean that the amount of activity per molecule needs to be the same. SsgA-like activity is thus defined as similar in kind, though not necessarily in amount. Other genes encoding such SsgA activity than the genes disclosed herein can be obtained without departing from the invention by applying routine hybridization and/or amplification techniques. Means methods for expressing such genes are well known in the art so that there is no need to go into detail here regarding cloning vectors, expression vectors, (inducible) promoters, enhancers, repressors, restriction enzymes, etc. etc. stability of the presence of the added SsgA-activity to the bacterium, in particular for application in large scale fementations, it is however preferred that the genetic information encoding the additional SsqA activity integrated into the host cell genome. In this case typically the genetic information will be in the form of DNA. However, neither RNA, heteroduplexes or even PNAs are excluded from the present invention as means to provide the additional genetic information to a microorganism. The invention is preferably applied in the field of filamentous bacteria, particular actinomycetes and most specifically streptomycetes. In these embodiments in particular it is preferred to apply ssgA genes derived from actinomycetes, especially from other actinomycetes than the one to in growth characteristics. This of course automatically the case in a bacterium that does not have SsgA activity to any significant amount itself. Using a gene from related organism enhances the compatibility of expression machinery of the host with the gene. Thus it is particularly preferred to provide a Streptomyces with an ssgA

10

30

(-like) gene from a different Streptomyces. SsgA genes are found in Streptomyces griseus, Streptomyces collinus, Streptomyces albus, Streptomyces goldeniensis and Streptomyces netropsis. It is preferred to provide Streptomyces strains not having such a gene with a gene from the earlier mentioned strains.

Typically said filamentous bacterium not having such a gene does not have significant endogenous SsgA-activity.

Itais useful to ensure that said additional SsgA-10 activity is inducible or repressible with a signal. In this way the growth characteristics of the bacteria can be modified at will. Of course the final goal of the present invention is to enhance the production of useful products by the microorganisms by modifying the microorganisms according to the invention. Useful products produced by or through microorganisms according to the invention include so called secondary metabolites, typically antibiotics or antitumour agents, but also immunosuppressive agents, hypocholesterolemic agents, enzyme inhibitors, antimigraine agents, herbicides, antiparasitic agents, ruminant growth 20 promoters, bioinsecticides, receptor (ant)agonists, hetreolgous proteins or even simple biomass. In the case of Streptomycetes such a useful product is typically antibiotic. It is thus therefore preferred according to the antibiotic producing strains invention to modify 25 Streptomyces, particularly those not harbouring an ssqA-like gene, with genetic information encoding ssgA activity. On the other hand the invention can also be very suitably applied to microorganisms expressing other Streptomycetes overexpressing proteins (or 30 heterologous homologous/endogenous proteins).

For ease of production it is preferred that the useful product, said antibiotic or said protein, is secreted by said bacterium. The protein to be expressed may very well

be a protein involved in the pathway of making a useful product such as an antibiotic, so that this production can be further enhanced on top of the improvement by the reduced fragmentation, etc. In that case it would be very suitable to 5 combine the two genes on one vehicle for introduction into the bacterium. The bacteria resulting from the methods according to the invention are of course also part of the invention. They have additional SsgA-activity (or are capable of expressing such activity) and they thereby will typically 10 have different growth characteristics than the unmodified microorganisms when said SsgA activity is present. Thus the invention also provides a filamentous bacterium obtainable by ac method according to invention. Preferred microorganisms according to the invention are actinomycetes and typically streptomycetes. As stated above it is an important goal of the present invention to improve fermentative production of makes museful products such as antibiotics. Thus the invention also provides a method for producing an antibiotic or a useful protein comprising culturing a filamentous bacterium 20 according to the invention and harvesting said antibiotic or protein from said culture. The advantages of the invention are most clear when the method of culturing is submerged figurature. The invention will be explained in more detail in the following experimental part.

25

30

Experimental procedures

Bacterial strains, culture conditions and plasmids

E. coli K-12 strains JM109 (Messing et al., 1981), and ET12567 (MacNeil, et al., 1992) were used for routine subcloning. The strains were grown and transformed by standard procedures (Sambrook et al., 1989); transformants were selected in L broth containing 1% (w/v) glucose, and ampicillin at a final concentration of 200 μg ml⁻¹. L broth

with 1% glucose and 30 μg ml⁻¹ chloramphenical was used to grow ET12567.

Streptomyces coelicolor A3(2) M145 and Streptomyces lividans 1326 (Hopwood et al., 1985) were used for transformation and propagation of Streptomyces plasmids. Protoplast preparation and transformation were performed as described by Hopwood et al. (1985). SFM medium (mannitol, 20 g l⁻¹; soya flour, 20 g l⁻¹; agar, 20 g l⁻¹, dissolved in tap water) is a modified version of that reported by Hobbs et al. (1989) and was used to make spore suspensions. R2YE (Hopwood et al., 1985) was used for regenerating protoplasts and, after addition of the appropriate antibiotic, for selecting recombinants.

For liquid culturing of *Streptomyces* we used YEME medium (Hopwood et al., 1985), Tryptone soy broth (Difco) containing 10% sucrose (designated TSBS), or standard minimal medium (MM; Hopwood et al.) with 1% mannitol as carbon source.

15

Strains used for screening of ssgA were Streptomyces albus G (ATCC 3004), Streptomyces ambofaciens (ATCC 23877), antibioticus Streptomyces (ATCC8663), Streptomyces clavuligerus (ATCC 27064), Streptomyces coelicolor M145, Streptomyces collinus (DSM 40733), Streptomyces fradiae (CBS goldeniensis Streptomyces (ATCC Streptomyces griseus (ATCC 23345), Streptomyces kasugaensis 25 (DSM 40819), Streptomyces lividans, Streptomyces mobaraensis Streptomyces (ATCC 25365), netropsis (formerly Streptoverticilium netropsis; ATCC 23940), Streptomyces ramocissimus (ATCC 27529), and the actinomycetes Nocardia lactamdurans (ATCC 27382), Planobispora rosea (ATCC 53773), Saccharopolyspora erythraea (NRRL 2338).

Plasmids pUC18 (Yanisch-Perron et al., 1985), pIJ2925 (Janssen and Bibb, 1993), and pSET152 (Bierman et al., 1992)

were used for cloning experiments. While pSET152 is a conjugative shuttle plasmid, in the experiments described in this study the plasmid and its derivatives were introduced by standard protoplast transformation.

pIJ486 (Ward et al., 1986) and the E. coli/Streptomyces shuttle vector pWHM3 (Vara et al.) as high copy-number vectors (approximately 50-100 copies per chromosome) in S. coelicolor. An expression vector, designated pWHM3-E, was constructed by cloning the 300 bp EcoRI/BamHI fragment containing the ermE promoter (Bibb et al., 1994) into pWHM3. Standard procedures were used to isolate plasmid DNA from E. coli (Sambrook et al., 1989), and to isolate plasmid and total DNA from Streptomyces (Hopwood et al., 1985).

15

20

25

30

PCR conditions

Polymerase chain reactions (PCRs) were performed in a minicycler (MJ Research, Watertown, MA), using Pfu polymerase (Stratagene, La Jolla, LA), and the buffer provided by the supplier, in the presence of 5% (v/v) DMSO and 200 mM dNTP. No additional Mg^{ft} was added to the reaction mixture. The following PCR program was used: 30 cycles of 45 seconds melting at 94°C, 1 minute annealing at 54°C, and 90 seconds extension at 72°C, followed by an additional 10 minutes at 72°C.

Constructs for expression of ssgA

A 750 bp DNA fragment containing the ssgA gene (Accession D50051) was amplified from the Streptomyces griseus chromosome by PCR, using primers ssg1 and ssg2 (Table 1). The PCR fragment was cloned as an EcoRI-BamHI fragment in pIJ2925, and further into pWHM3, pWHM3-E, and pSET152, resulting in pGWS1, pGWS2, pGWS3, and pGWS4, respectively

(Table 1). For pGWS1 and pGWS3, see also Figure 1. The S. coelicolor strain with pGWS4 integrated in the attP site on the chromosome was designated S. coelicolor GSA1. For pGWS1, pGWS3, and pGWS4 we also made derivatives in which the upstream region of S. griseus ssgA was replaced by that of S. ramocissmus tuf1 (Vijgenboom et al., 1994), which is known to be very efficiently recognized by ribosomes and hence typically results in higher expression; these were designated pGWS1-SD, pGWS3-SD, and pGWS4-SD, respectively.

10

15

20

25

30

Southern hybridization and probes

Genomic DNAs used for Southern analysis were isolated according to the method described by Hopwood et al. (1985). For high-resolution hybridization experiments, to investigate the presence of ssqA in various actinomycetes, genomic DNA was digested with the appropriate enzymes and separated electrophoretically on a 0.7% agarose gel in TAE buffer, using the Gibco BRL 1 kb ladder as DNA size markers. Agarose gels were pretreated and subsequently blotted on Hybond-N+ (Amersham) using 20x SSC buffer as the nylon membranes transfer buffer, basically according to Sambrook et (1989). Hybridization and washing conditions were described previously (van Wezel et al., 1991). Stripping of blots was done by 30 minutes incubation in 0.4 N NaOH at 65°C and subsequent incubation in 0.1x SSC/0.25 M Tris (pH 6.5). The total removal of the probe was checked by overnight exposure of an X-ray film.

For recognition of ssgA in Southern hybridization experiments the 580 bp insert from pGWS5 was [32 P]-labelled by the random-prime method (Feinberg and Vogelstein, 1983).

Computer analysis

The BLAST search engines BlastN, BlastP, and BlastX (Altschul et al., 1990) were used to perform database searches, and the Wisconsin GCG Package (Devereux et al., 1984) for sequence alignments and protein analysis.

s and but the base of Results had be

SsgA is a unique protein that does not belong to any

THE DRIVE AND THE EXCLUSIVE STORY OF THE

Carrier and a constant confineer as a second of the confineer and the

Extensive searches with *S. griseus* SsgA of both the translated nucleotide database and the protein database using the BLAST search engines BLASTX and BLASTP resulted in one relevant hit, namely a partial sequence of *Streptomyces albus*15 G DNA (Accession M28303) that apparently encodes part of SsgA. This DNA was identified upstream of a ß-lactamase gene (Dehottay et al., 1987), and apparently encodes 67 residues of a putative protein with 86% as identity to as 18-84 of *S. griseus* SsgA. The lack of the C-terminal half of the gene suggests that the cloning of this *ssgA* homologue was probably coincidental and the result of a cloning artifact. The cloning and sequencing of the complete gene is described below.

25 Cloning of S. griseus ssgA by PCR

30

The sequence of *S. griseus ssgA* was published by Kawamoto and Ensign (1995b), and deposited in the EMBL/GENBANK database (D50051). In a recent update the translational start codon was proposed 30 nt downstream of the originally indicated start codon. This ambiguity does not influence the outcome of our experiments. Based on protein electrophoresis (SDS PAGE) experiments using over-expressed SsgA, we believe that the second (further downstream located)

ATG triplet represents the correct translational start codon (data not shown).

The 750 bp DNA fragment generated by PCR amplification of S. griseus chromosomal DNA using oligonucleotides ssgl and ssg2 was cloned into pIJ2925, resulting in pGWS1 (Table 1). Restriction site and sequence analysis confirmed that the fragment indeed contained ssgA.

and the figure of a five contraction of the part of the contraction of

Southern hybridization reveals ssgA in a limited number of streptomycetes

Genomic DNAs isolated from several actinomycetes (see legend to Fig. 2) was digested with BamHI and PstI, submitted to agarose gel electrophoresis and hybridised with the 580 bp from pGWS5 harbouring S. griseus conditions of low stringency to identify all genes with at least remote similarity to ssgA. One hybridising band was observed in the lanes containing S. collinus, S. albus, S. goldeniensis, and S. griseus genomic DNAs, and two bands of equal intensity in the lane containing S. netropsis DNA (Fig. 20 2). We failed to detect a band corresponding to ssgA in all other Streptomyces species, including S. coelicolor and S. lividans, in contrast to a previous Southern analysis by Kawamoto and Ensign (1995b), who used a probe that included ssgA flanking sequences. The duplicity of the signal 25 corresponding to ssgA in S. netropsis was due to a BamHI restiction site in the gene, as can be deduced from the DNA sequence. We also could not detect an ssgA homologue in any of the other actinomycetes checked, namely Nocardia lactamdurans, Planobispora rosea, and Saccharopolyspora

30 erythraea.

10

Cloning and sequencing of ssgA homologues from other streptomycetes

Genomic DNA fragments harbouring ssgA homologues from three streptomycetes, namely S. albus, S. goldeniensis, and 5 S. netropsis, were amplified by PCR, susing oligonucleotides ssg3 and ssg4. These fragments were cloned as EcoRI/BamHI fragments into pIJ2925, and the DNA sequence was determined. Table 2 shows the similarities of the ssqA genes and the deduced amino acid sequences. Interestingly, the S. netropsis and S. griseus ssgA gene products share more than 86% identical amino acids (90% similar), which is high in comparison to 79% (85%) for S. goldeniensis SsqA and, strikingly, a poor 63% (71%) for S. albus SsgA.

Section 1985

15 S. griseus and S. netropsis sporulate in liquid ASSESSMENT Cultures () A Company of the fine for a company of the

gridated and the state of the

The morphology of the streptomycetes actinomycetes discussed in this paper was checked by light microscopy. To this purpose, the strains were grown in 20 complex (TSBS) or minimal (MM) liquid medium for three days, and growth characteristics monitored. From these experiments it appeared that only S. griseus and S. netropsis produced abundant spores in liquid cultures, while S. goldeniensis and S. collinus showed unusual thickening of the tips of the hyphae, but failed to sporulate under the chosen conditions. 25 Interestingly, while S. griseus sporulated only in MM, as was already reported by Kendrick and Ensign (1983) & S. netropsis sporulated abundantly in TSBS as well as in MM. We believe that the relation between sporulation and (the amino acid sequence of) SsgA is of particular interest.

Expression of ssgA in S. coelicolor M145 results in reduced branching of the hyphae and fragmented growth

The insert of pGWS1 was cloned into pWHM3 and pWHM3-E, multicopy shuttle vectors that replicate in *E. coli* and *Streptomyces*. The resulting plasmids pGWS2 and pGWS3 (Table 1) were introduced into *S. coelicolor* M145 and correct recombinants were selected by checking the insert lengths of the plasmids. In a control experiment we used pWHM3-E transformants.

10 Transformants containing pWHM3-E (without ssgA) or pGWS2 showed little or no altered morphology in the complex liquid media TSBS, YEME, nor in minimal medium (MM), as judged by phase-contrast microscopy (Fig. 3A). However, hyphae of transformants containing pGWS3 showed strongly reduced branching in complex and minimal medium cultures, 15 resulting in clearly less dense mycelial lumps (Fig. 3B). The vegetative hyphae not only show limited branching, but many of the branches are less than a micron in length. When pGWS3-SD was used instead of pGWS3, the effect was even stronger, 20 with small fragments appearing after approximately 30 thrs, which increased over time (Fig. 4). While MM cultures of S. coelicolor typically result in very large mycelial lumps that sediment rapidly (virtually all mycelium precipitates within one minute when shaking was stopped), MM cultures containing 25 pGWS3-SD transformants showed significantly sedimentation rates, with the majority of the mycelium failing to sediment within five minutes after shaking of the cultures was stopped.

Expression of chromosomally-integrated ssgA also results in fragmented growth

The insert of pGWS3 and pGWS3-SD was cloned in pSET152, a conjugative *E. coli/Streptomyces* shuttle vector,

resulting in pGWS4 and pGWS4-SD, respectively. These plasmids were, introduced sinto S. coelicolor M145 by standard protoplast transformation, and transformants selected by overlay of the transformation plates with 5 Chromosomal integration was checked by Southern analysis, and presence of the complete gene confirmed by PCR using oligonucleotides ssgl and ssgl. The pGWS4 and pGWS4-SD integrants were designated GSA1 and GSA2. S. coelicolor M145 harbouring pSET152 without ssgA was used as control strain.

While recombinants containing pSET152 displayed wild-10 ... type phenotype, with large mycelial lumps and very few smaller fragments, GSA1 showed limited branching, while the phenotype of GSA2 is much similar to that of S. coelicolor harbouring pGWS3-SD, sowith strongly limited branching and 15 fragmented growth (Fig. 4). This shows that S. griseus ssgA integrated in the S. coelicolor chromosome can be expressed of the decate and levely high; enough to callow, fragmentation of coelicolor mycelium in complex and minimal liquid cultures.

20.8 gas a High-level (expression of assgA in other actinomycetes

in the effect of the work of the effect of Server that other with being being

30

The ssgA expression vectors pGWS3-SD and pGWS4 were introduced in S. slividans, S. clavuligerus, and Sacch. erythraeato to testouthes effects of SsgA on the amorphology of strains other than S. coelicolor. Expression in S. lividans 25 using pGWS3-SD or pGWS4 led to a phenotype much similar to that of S. coelicolor harbouring the same plasmids, as was expected since Similaridans and Simcoelicolor are strongly related streptomycetes. Interestingly, expression of SsgA in both S. clavuligerus and Sacch. erythraea also resulted in reduced branching and increased fragmentation in liquid cultures, even though morphology of these strains is different from that of S. coelicolor.

Thus, it appears that overproduction of SsgA has a strong effect on mycelium morphology in submerged cultures of not only S. coelicolor, but also of S. lividans, S. clavuligerus, and Sacch. erythraea, with the vegetative

5 hyphae showing restricted branching. Furthermore, the ageing cultures showed an increasing degree of fragmentation, resulting in higher culture densities and lower viscosity of recombinant streptomycetes expressing SsgA. Comparison of the phenotypes of the two categories of Streptomyces strains,

10 namely those possessing ssgA and those lacking ssgA, is currently in progress, and could give us more insight into the role of SsgA in Streptomyces physiology.

en en la compara de la comp La compara de la compara del la com

and the control of the second temperature of the control of the co

and Andrew Johnson (1968) the analysis was a significant provide and the season of the

n de la filipia de la companya de l Companya de la compa

and the substitution of the control of the control

Figure	leger	nds

20

Figure 1. Some of the ssgA constructs. Arrows show direction of ssgA. P_{ermE}, ermE

promoter; P_{T7}, T7 promoter. Solid lines represent ssgA DNA, broken lines
represent plasmid DNA.

400000 360

and parties of the Secretary of the professional

NOTE RESIDENCE OF STOLE

ing transcript to gradate the little of a

- Figure 2. Southern hybridization for the detection of ssgA in actinomycetes. All numbered lanes contain BamHI/PstI-digested chromomal DNA. Marker lanes

 (M) contain 1 kb DNA ladder. Blots were hybridized with the 580 bp insert from pGWS5 as probe, and subsequently with a small amount of radioactively labelled 1 kb ladder.
- A. Lanes: 1. S. coelicolor 2. S. lividans 1326 3. S. lividans TK24 4. S. griseofuscus 5.

 S. fradiae 6. S. ramocissimus 7. S. collinus 8. S. kasugaensis 9. S. antibioticus 10.

 Sacch. erythraea 11. N. lactamdurans 12. P. rosea 13. S. griseus
 - B. Lanes: 1. S. albus 2. S. ambofaciens 3. S. coelicolor 4. S. clavuligerus 5. S. collinus 6. Sacch. erythraea 7. S. goldeniensis 8. S. mobaraensis 9. S. netropsis 10. P. rosea

"这些大人,那麽"(thad bill 14 多字

- **Figure 3.** Phase-contrast microscopy of *S. coelicolor* M145 containing pGWS2, pGWS3, and pGWS3-SD.
- Figure 4. Phase-contrast microscopy of *S. coelicolor* M145 containing chromosomallyintegrated pGWS4.
 - Figure 5. Sequences of different ssga genes and proteins from different strains and oligonucleotides.

Oligonucleotides

		A second of the	•
	primer	n North Carlot, Christian de Autorio grégoriem de la companya de l	Nucl. Pos.
5	ssg1	5' <u>GGCGAATTC</u> GAACAGCTACGTGGCGAAGTCGCCA 3' <i>Eco</i> RI	-194/-170
10		5' <u>GTGGGATCC</u> GTGCTCGCGGCGCTGGTCGTCTC 3' BamHI 5' <u>GGGAATTCCAT</u> ATGCGCGAGTCGGTTCAAGCA 3'	E g tif
	5560	EcoRI NdeI	-30/-10
15	ssg4 Plasmids	5' CCGGTCAGCCGGCGTTCTGCTCCTC 3'	+412/388
20	Bibb,	Derivative of pUC19, with Bg/II sites flanking the slightly altered multiple cloning site.	Janssen and 1993
,		Multi-copy E. coli/Streptomyces shuttle vector. Carries thiostrepton resistance marker	Vara et al.
2,5	рWНМ3-Е	pWHM3 with the 300 bp fragment containing the constitutive ermE promoter for gene expression	this study
30	pSET152	E. coli/Streptomyces shuttle vector that allows integration in the _C31 attachment site on the Streptomyces chromosome. Carries apramycin resistance marker.	Bierman et al., 1992
	pGWS1	pIJ2925 containing the 750 bp ssgA PCR (ssg1/ssg2) product	this study
35	pGWS1-SD	pGWS1 with the upstream region of ssgA replaced by nt -1/-70 of S. ramocissimus tuf1	this study
	pGWS2	pWHM3 containing the EcoRI/HindIII insert from pGWS1	this study
40	pGWS3	pWHM3-E containing the BglII/HindIII insert from pGWS1	this study
	pGWS3-SD	pWHM3-E containing the BglII/HindIII insert from pGWS1-SD	this study
	pGWS4	pSET152 containing the EcoRI/PstI insert from pGWS3	this study

pGWS4-SD pSET152 containing the EcoRI/PstI insert from pGWS3-SD

and a such as ever **ou**rse a major called by the property of

this study

5

pIJ2925 containing the 580 bp ssgA PCR (ssg3/ssg2) product cloned EcoRI/BamHI.

Table 1. Oligonucleotides and ssgA constructs. Nucleotide positions refer to the location of the primers in respect to the first nucleotide (+1) of the ATG translational start codon of ssgA. Underlined sequences indicate non-homologous sequences added to create restriction sites (in italics) at the ends of the PCR fragments.

is not the Carlotter for the control of the Carlotter for the Market of the Market of

15

 $\lambda_1 = 1 + \frac{1}{2} \cdot P$

	S. albus	S. goldeniensis	S. griseus	S. netropsis		
S. albus	х	75.2	74.5	72.3		
S. goldeniensis	71.3 (75.7)	X	77.5	75.7		
S. griseus	66.2 (71.3)	78.7 (85.3)	X	83.3		
S. netropsis	63.2 (70:6) գունչ	77.9 (83.8)	(90.4)	X		

Table 2. DNA and deduced protein sequence homologies of ssgA homologues. Above the diagonal: DNA sequence identities (%). Below the diagonal: protein sequence identities (similarities between brackets).

and the control of the second section of the control of the contro

the Commercial Address to the control of the contro

and the state of t

Japan Gariff Marian Carrier Figure

and the second of the second o

o de la filo de de la filosofia. O de la filosofia de la filosofia

References

Bibb, M.J., White, J., Ward, J.M., and Janssen, G.R. (1994) The mRNA for the 23S rRNA methylase encoded by the *ermE* gene of *Saccharopolyspora erythraea* is translated in the absence of a conventional ribosome-binding site. *Mol. Microbiol.* **14**: 533–45.

The property of the second

Bierman, M., R. Logan, K. Obrien, E.T. Seno, R.N. Rao, and Schoner, B.E. (1992) Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to *Streptomyces* spp. *Gene* 116: 43-49.

that the rest independent of the

Chater, K.F., and Losick, R. (1996) The mycelial life-style of *Streptomyces coelicolor* A3(2) and its relatives. In J.H. Shapiro and M. Dworkin (ed.), Bacteria as Multicellular Organisms. Oxford University Press, New York.

15

25

10

Dehottay, P., Dusart, J., De Meester, F., Joris, B., van Beeumen, J., Erpicum, T., Frere, J.-M., and Ghuysen, J.-M. (1987) Nucleotide sequence of the gene encoding the *Streptomyces albus* G \(\mathcal{B}\)-lactamase gene. Eur. J. Biochem. **166**: 345-350.

Devereux, J., Haeberli, P., and Smithies, O. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* 12: 387-395.

Ensign, J.C. (1988) Physiological regulation of sporulation of *Streptomyces griseus*. In Y. Okami, T. Beppu, and H. Ogawara (eds.), Biology of Actinomycetes 1988, pp. 308-315. Tokyo, Japan Scientific Societies Press.

Feinberg, A.P., and Vogelstein, B. (1983) A technique for radiolabeling of DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132:** 6-13.

Hobbs, G., Frazer, C.M., Gardner, D.C.J., Flett, F., and Oliver, S.G. (1989) Dispersed growth of *Streptomyces* in liquid culture. *Appl Microbiol Biotechnol* 31:272-277.

Hopwood, D.A., Bibb, M.J., Chater, K.F., Kieser, T., Bruton, C.J., Kieser, H.M., Lydiate, D.J., Smith, C., Ward, J.M., and Schrempf, H. (1985) Genetic manipulation of *Streptomyces*: a laboratory manual. John Innes Foundation, Norwich, U.K.

Janssen, G.R., and Bibb, M.J. (1993) Derivatives of pUC18 that have *BgIII* sites flanking a modified multiple cloning site and that retain the ability to identify recombinant clones by visual screening of *Escherichia coli* colonies. *Gene* 124: 133-134.

五个台灣地區或多 新原 医牙内溃疡 医二类反应 医神经病性病 医二氏硷

សាទីស ទៅ ទីក្រុម **មួស** មួស មាន ប្រជាធិបាល ការប្រ

Kawamoto, S., and Ensign, J.C. (1995a) Isolation of mutants of Streptomyces griseus that sporulate in nutrient rich media: cloning of DNA fragments that suppress the mutations. Actinomycetologica 9: 124-135.

stated that the second discovering and the first of the second of the second se

Alleger 1987 All Bright Collaboration Classes Agency Control of the Collaboration Street, and

Kawamoto, S., and Ensign, J.C. (1995b) Cloning and characterization of a gene involved in regulation and sporulation and cell division in *Streptomyces griseus*. Actinomycetologica 9: 136-151.

Kawamoto, S., Watanabe, H., Hesketh, A., Ensign, J.C., and Ochi, K. (1997) Expression of the ssgA gene product, associated with sporulation and cell division in Streptomyces griseus. Microbiology 143: 1077-1086.

20 P. C. C. P. C. C. B. C. S. C.

1

Kendrick, K., and Ensign, J.C. (1983) Sporulation of Streptomyces griseus in submerged culture. J. Bacteriol. 155: 357-366.

A A ASSET OF COMMENTS OF THE SECOND

a soop to ordering a rought of the total ledge to

Lutkenhaus, J., and Addinall, S.G. (1997) Bacterial cell division and the Z ring. Annu Rev. Biochem. 66: 993-116.

MacNeil, D.J., Gewain, K.M., Ruby, C.L., Dezeny, G., Gibbons, P.H., and MacNeil, T. (1992) Analysis of *Streptomyces avermitilis* genes required for avermectin biosynthesis utilising a novel integration vector. *Gene* 111: 1-68.

30

25

McCarthy, A.J., and Williams, S.T. (1992) Actinomycetes as agents of biodegradation in the environment - a review. *Gene* 115: 189-192.

McCormick, J.R., Su, E.P., Driks, A., and Losick, R. (1994) growth and viability of Streptomyces coelicolor mutant for the cell division gene ftsZ. Mol. Microbiol. 14: 243-254.

的。一個的**建位 20**00年11年

for I make the

Messing, J., Crea, R., and Seeburg, P.H. (1981) A system for shotgun DNA sequencing. Nucleic Acids Res 9: 309-321.

to the first of the second

Miyadoh, S. (1993) Research on antibiotic screening in Japan over the last decade: a producing microorganisms approach. *Actinomycetol* 7: 100-106.

10

正大性 医催化剂抗原

Redenbach, M., Kieser, H.M., Denapaite, D., Eichner, A., Cullum, J., Kinashi, H., and Hopwood, D.A. (1996) A set of ordered cosmids and a detailed genetic and physical map for the 8 Mb *Streptomyces coelicolor* A3(2) chromosome. *Mol Microbiol*.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Strohl, W.R. (1992) Compilation and analysis of DNA sequences associated with apparent streptomycete promoters. *Nucleic Acids Res* 20: 961-974.

20

van Wezel, G.P., Vijgenboom, E., and Bosch, L. (1991) A comparative study of the ribosomal RNA operons of *Streptomyces coelicolor* A3(2) and sequence analysis of *rrnA*. *Nucleic Acids Res* 19: 4399-4403.

- Vara, J., Lewandowska-Skarbek, M., Wang, Y.-G., Donadio, S., and Hutchinson, C.R. (1989) Cloning of genes governing the deoxysugar portion of the erythromycin biosynthesis pathway in *saccharopolyspora erythraea* (Streptomyces erythreus). J. Bacteriol. 171: 5872-5881.
- Vijgenboom, E., Woudt, L.P., Heinstra, P.W.H., Rietveld, K., van Haarlem, J., van Wezel, G.P., Shochat, S., and Bosch, L. (1994) three tuf-like genes in the kirromycin producer Streptomyces ramocissimus. Microbiology 140: 983-998.

Ward, J.M., Janssen, G.R., Kieser, T., Bibb, M.J., Buttner, M.J., and Bibb, M.J. (1986) Construction and characterisation of a series of multi-copy promoter-probe plasmid vectors for *Streptomyces* using the aminoglycoside phosphotransferase gene from Tn5 as indicator. *Mol Gen Genet* 203: 468-475.

Yanish-Perron, C., Vieira, J., and Messing, J. (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13 mp18 and pUC19 vectors. *Gene* 33: 103 119.

and the exploration of a street of everygen glabilities.

and the second of the second of the second

in 1903 – The Control of the Control

and the same of the control of the c

a said that they

しゃ しゅうしん といれ しょくしゅ コンディック さんりん

Conference of the Conference o

Water the State of the State of

The special control of the Signature

ALL STEEDERS AND MARKETONES AND CONTRACTOR OF THE STEED AND STEED AND STEEDERS.

faction with a property of

Claims

- 1. A method for producing a filamentous bacterium showing reduced branching during growth, particularly growth in a liquid medium, comprising providing such a bacterium with the capability of having or expressing heterologous SsgA-activity, which activity in *Streptomyces Griseus* is encoded by an ssgA gene having at least the sequence
- 1 ATGCGCGAGTCGGTTCAAGCAGAGGTCATGATGAGCTTCCTCGTCTCCGA
- 51 GGAGCTCTCGTTCCGTATTCCGGTGGAGCTCCGATACGAGGTCGGCGATC

10

- 101 CGTATGCCATCCGGATGACGTTCCACCTTCCCGGCGATGCCCCTGTGACC
- 151 TGGGCGTTCGGCCGCGAGCTGCTGGACGGGCTCAACAGCCCGAGCGG
- 15 201 CGACGGCGATGTGCACATCGGCCCGACCGAGCCCGAGGGCCTCGGAGATG

 - 301 ACGGCACCGCTGGTGGCGTTCCTCGACCGGACGGACAAGCTCGTGCCGCT

20

- 351 CGGCCAGGAGCACACGCTGGGTGACTTCGACGGCAACCTGGAGGACGCAC
- 401 TGGGCCGCATCCTCGCCGAGGAGCAGAACGCCGGCTGA.
- 2. A method for producing a filamentous bacterium showing enhanced fragmentation during growth, particularly growth in a liquid medium, comprising providing such a bacterium with the capability of having or expressing heterologous SsgA-activity, which activity in Streptomyces Griseus is encoded by an ssgA gene having the sequence

- 1 ATGCGCGAGTCGGTTCAAGCAGAGGTCATGATGAGCTTCCTCGTCTCCGA
- 51 GGAGCTCTCGTTCCGTATTCCGGTGGAGCTCCGATACGAGGTCGGCGATC
- 5 101 CGTATGCCATCCGGATGACGTTCCACCTTCCCGGCGATGCCCCTGTGACC

Cartination in the control of the cartinate and the control of the

- 151 TGGGCGTTCGGCCGCGAGCTGCTGCTGGACGGGCTCAACAGCCCGAGCGG
- 201 CGACGGCGATGTGCACATCGGCCCGACCGAGCCCGAGGGCCTCGGAGATG

10

- 301 ACGGCACCGCTGGTGGCGTTCCTCGACCGGACGGACAAGCTCGTGCCGCT
- 15 351 CGGCCAGGAGCACACGCTGGGTGACTTCGACGCCAACCTGGAGGACGCAC
 - 401 TGGGCCGCATCCTCGCCGAGGAGCAGAACGCCGGCTGA

· 我只要是我的一直看到一直在"大大大大"的"我们的"。 (1997) [187]

20 3. A method according to claim 1 or 2, whereby said additional SsgA-activity is provided by transfecting or transforming said filamentous bacterium with additional genetic information encoding said activity.

- 4. A method according to claim 3, whereby said additional genetic information comprises an ssgA gene or a derivative or a fragment thereof encoding similar SsgA-activity.
 - 5. A method according to claim 4, whereby said ssgA gene is derived from an actinomycete.
- 6. A method according to claim 4, whereby said gene is derived from a streptomycete.
 - 7. A method according to claim 5, whereby said gene is derived from Streptomyces griseus, Streptomyces collinus, Streptomyces albus, Streptomyces goldeniensis or Streptomyces netropsis.

- 8. A method according to any one of claims 3-7, whereby said additional genetic information is integrated into the bacterial genome:
- 9. A method according to any one of claims 3-8, whereby said additional genetic information is part of an episomal element.
 - 10. A method according to any one of the aforegoing claims, whereby said filamentous bacterium does not have significant endogenous ssgA-activity.
- 10 11. A method according to any one of the aforegoing claims wherein said ssgA-activity is inducible or repressible with a signal.
 - 12. A method according to any one of the aforegoing claims wherein said filamentous bacterium is an Actinomyces.
- 15 13. A method according to claim 12, wherein said bacterium is a Streptomyces.
 - 14. A method according to any one of the aforegoing claims whereby said bacterium produces a useful product.
- 15. A method according to claim 14 wherein said useful 20 product is an antibiotic.
- 16. A method according to claim 14, whereby said useful product is a protein.
 - 17. A method according to claim 16 whereby said protein is heterologous to said bacterium.
 - 25 18. A method according to claim 16 or 17, whereby said protein is expressed from a vector encoding said protein present in said bacterium.
 - 19. A method according to claim 16, 17 or 18, whereby said protein is secreted by said bacterium.
 - 30 20. A filamentous bacterium obtainable by a method according to any one of the aforegoing claims.
 - 21. A filamentous bacterium according to claim 20, which is an actinomycete, preferably a *Streptomyces*.

- A method for producing an antibictic or a useful protein comprising culturing a filamentous bacterium according to claim 19 or 21 and harvesting said antibiotic or protein from said culture.
- A method according to claim 22 whereby said culturing 5 23. is submerged culture.

The later of the state of the s TO BROWN THE POPULATIONS

The state of the s

1000

1996年 - 1996年 a 21 (21 to 1) in the responsibility of the collection of the coll

Buy to see with the thirty of the

ANTO ANTO MAY ANTO ANTO ANTO ANTO The Common Street Common

Electronic transfer examination of the control of

the first of the second with the second seco

SEQUENCE LISTING

Carrier and analysis of

each arm because might

(1) GENERAL INFORMATION:

- (i) APPLICANT:
 - (A) NAME: Rijksuniversiteit Leiden
 - (B) STREET: Wassenaarseweg 72
 - (C) CITY: Leiden
- (E) COUNTRY: The Netherlands
 (F) POSTAL CODE (ZIP): 2300 RA
- (ii) TITLE OF INVENTION: Reducing branching and enhancing fragmentation in culturing filamentous microorganisms
- (iii) NUMBER OF SEQUENCES: 12
- (iv) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)
- (2) INFORMATION FOR SEQ ID NO: 1:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 408 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (iii) HYPOTHETICAL: NO
 - (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Streptomyces griseus
 - (B) STRAIN: ATTC 23345
 - (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1..408
 - (D) OTHER INFORMATION: /product= "SsgA" /gene= "ssgA"
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ATG AGC TTC CTC GTC TCC GAG GAG CTC TCG TTC CGT ATT CCG GTG GAG Met Ser Phe Leu Val Ser Glu Glu Leu Ser Phe Arg Ile Pro Val Glu 1 10

CTC CGA TAC GAG GTC GGC GAT CCG TAT GCC ATC CGG ATG ACG TTC CAC Leu Arg Tyr Glu Val Gly Asp Pro Tyr Ala Ile Arg Met Thr Phe His

20

96

																CTG		144
	Leu	Pro		Asp	Ala	Pro	Val		\mathtt{Trp}	Ala	Phe	Gly		Glu	Leu	Leu		
			35					40					45					
	ama	C T C	~~~		7 7 C	700	aaa	700	ccc				. · · ·		אשמ	000	7 25	. 1.00
																GGC Gly		192
	neu	50	_						_	_	_	_			TIE	-		
		50			111		. 55		;	1 . A		, ,		•		· · · ·		•
	CCG	ACC	GAG	CCC	GAG	GGC	CTC	GGA	GAT	GTC	CAC	ATC	CGG	CTC	CAG	GTC		240
																Val.	e sa j	
	65				•	70					75	:	•			80		
•																		
																GCG		288
	GIĀ	Ala	Asp	Arg	85	ьеи	PHE	Arg	Ala	90	TIIL	на	PLO	Leu	95	AIA		
					Ų,					,,					,,			
	TTC	CTC	GAC	CGG	ACG	GAC	AAG	CTC	GTG	CCG	CTC	GGC	CAG	GAG	CAC	ACG		336
	Phe	Leu	Asp	Arg	Thr	Asp	Lys	Leu	Val	Pro	Leu	Gly	Gln	Glu	His	Thr		
				100					105		÷ }	1 4 3		110	٠,	100	Mili	
	OTTC	aam	aza	mma	aza.	000	220	CTTC	CAC	CAC	CCN	ama	aaa	cca	N ITTO	ama		204
		Gly													•	CTC	ţ.i.	384
	пси	GLY	115	LIIC	ASD.	Gry	ADII	120	01u	HDD.	71.00		125			БСС		
	GCC	GAG	GAG	CAG	AAC	GCC	GGC	TG						1.0				408
	Ala	Glu	Glu	Gln	Asn	Ala	Gly											
		130					135			. :				4.5	٠.		::;	
													•			18 4		
														215			1 47	
	(2)	INFO	RMAT	NOI	FOR	SEQ	ID 1	10: 2	: ,	1.10	- 0		1					
												,	. :		. ,	÷ .		
		(RACTI											
			-	-			35 an 10 ac			ıs					·	. 1	1.173	
							line					1	 . :	i - jo.	. ":	,		
•										. :					inder Aussiania Maria			
		(ii)	MOL	ECUI	E TY	PE:	prot	ein	•			· · · · · · · · · · · · · · · · · · ·			•			
		, .,										·						
		(XI)	SEÇ	MENC	E DE	SCRI	IPTIC	N: E	EQ 1	טא ט.): 2:	4. 3						
	Met	Ser	Phe	Leu	Val	Ser	Glu	Glu	Leu	Ser	Phe	Arq	Ile	Pro	Val-	Glu		
	1				5					10					15			
						·	·	•										
	Leu	Arg	_													His		
	٠٠.		.: .	_ 20			÷ .		25		100	7		30.		-		.*
	T on	Pro	Glar	7.00	λla	Pro	 37:a T	Thr	Trans.	: Δ] a	Dhe	Glaz	A ror	Glu	T.011	Len	10.0	45
	Leu	PIO	35		Ата	FIO	val	40	ırp	n'a	FIIC	GLY	45	GLU	пец	Beu		
													- -					
-	Leu	Asp	Gly	Leu	Asn	Ser	Pro	Ser	Gly	Asp	Gly	qaA	Val	His	Ile	Gly		
		50					55				•	60						

Pro Thr Glu Pro Glu Gly Leu Gly Asp Val His Ile Arg Leu Gln Val 65 70 80
Gly Ala Asp Arg Ala Leu Phe Arg Ala Gly Thr Ala Pro Leu Val Ala
్
Phe Leu Asp Arg Thr Asp Lys Leu Val Pro Leu Gly Gln Glu His Thr
100 105 110 24 9 1 1 1 2 4 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
Leu Gly Asp Phe Asp Gly Asn Leu Glu Asp Ala Leu Gly Arg Ile Leu
115 120 125
Ala Glu Glu Gln Asn Ala Gly 1 12 5 12 12 12 12 12 12 12 12 12 12 12 12 12
ನಗಳು ಬಂದು ನೀಡು ಮುಂದು ಶಾಲಕ ಅವರ ತನ್ನಾಗಿಗಳು ಕರ್ಕನ್ನು ಗಳುಗೆ ಅವರ ಅವರ ಕೊಂದು ಬಂದು ಗಳುಗೆ ಎಂದು ಕ್ರಾಂಡಿಗೆ ನೀಡುಕೊಂಡು ಕೂಡು ಅವರ ಕ್ರಾಂಡಿಗಳು ಕೊಂದು ಕೊಂಡು ಕೊಂದು ಕೊಂದು ಕೊಂದು ಕೊಂದು ಕೊಂದು ಕೊಂದು ಕೊಂದು ಕೊಂದು ಕೊಂದು
(2) INFORMATION FOR SEQ ID NO: 3:
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 408 base pairs (B) TYPE: nucleic acid
(C) STRANDEDNESS: double (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(iii) HYPOTHETICAL: NO
<pre>(vi) ORIGINAL SOURCE: (A) ORGANISM: Streptomyces albus G (B) STRAIN: ATCC 3004</pre>
(ix) FEATURE:
(A) NAME/KEY: CDS (B) LOCATION: 1408
(D) OTHER INFORMATION: /product= "SsgA"
/gene= "ssgA"
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:
ATG AGC TTC CTC GTC TCC GAG GAG CTC GCC TTC CGC ATC CCG GTG GAG 48
Met Ser Phe Leu Val Ser Glu Glu Leu Ala Phe Arg Ile Pro Val Glu
1
CTG CGG TAC GAG ACC GTC GAT CCG TAC GCG GTG CGG CTG ACG TTC CAC 96
Leu Arg Tyr Glu Thr Val Asp Pro Tyr Ala Val Arg Leu Thr Phe His
20 25 30
25

					GAC Asp												192
	50	-				-55	٠.		د این ا اداری		60						. *
					GCC Ala 70						Ile				GTC Val 80		240
															GCC Ala	en in Na	288
					GAC Asp					Leu	Gly	Ser	Glu	Arg			336
					AGC Ser			Asp		Ala	Leu	Asn 125	Arg		Leu	(S	384
					GCC Ala				1.77					Na. N		. *	408
(2)	INF	ORMAT	rion	FOR	SEQ	ID I	7O: 4	4:					*		1.		
		(i) s	SEOU	ENCE	CHAI	RACTI	ERIS'	rics	:	•							
		(<i>1</i>	4) LI 3) T	engti YPE :	CHAI H: 13 amii OGY:	35 at	mino cid ear	acio	is		21 21 F 1	. *	, to king Toward	2 (1.27 e) 3 (1	5, 1, 2 1, 5 1, 5	+24	
	(ii)	() (1) (1) (1)	A) LI B) T D) T LECUI	ENGTI YPE: OPOLO	H: 13 amin OGY: YPE:	35 at no ac line pro	mino cid ear tein	acio	is	N.	21 11 ft 1 12 ft 14 ft 14 ft 14 ft		, Alles Nove d Davie	e kut e til uttilet N	5, 5 a 4, 5 a 4, 5	•24 •	
	(ii) (xi)	(I (I (I MOI SEG	LECUI	ENGTI YPE: DPOLO LE TI	H: 13 amin DGY: YPE:	35 amo ao lino prof	mino cid ear tein	acio	ds ID No); /4	27 10 \$ 1 10 \$2.0 10 \$ 2 \$ 1 \$ 2			(3.0%) (4.0%) (4.0%) (4.0%) (4.0%)			
Met 1	(ii) (xi) Ser	(I (I (I MOI SEQ	A) LH B) TO C) TO CLECUI QUENO LEU	ENGTI YPE: OPOLO LE T' CE DI	H: 13 amin DGY: YPE:	35 and a control of the control of t	nino cid ear tein ON:	acio	is ID NO Ala	0: 4 Phe	ing Tight Tight Tight	Ile	Pro	(3.0%) (4.0%) (4.0%) (4.0%) (4.0%)	Glu		100 miles (100 miles (
1	(ii) (xi) Ser	(I (I (I) MOI SEG Phe	A) LH B) T C) TC LECUI QUENC Leu Glu 20	ENGTI YPE: OPOLO LE T' CE DI Val 5	H: 13 amin DGY: YPE: ESCR: Ser Val	35 am no ac line prof	mino cid ear tein ON: Glu Pro	acio SEQ Leu Tyr 25	ID NO Ala 10 Ala	O: 4 Phe Val	Arg	Ile Leu	Pro	Val	Glu		
1 Leu	(ii) (xi) Ser	(I) (II) (II) (II) (II) (II) (II) (II)	A) LI B) TO C) TO LECUI QUENO Leu Glu 20 Asp	ENGTI YPE: DPOLO LE T CE DI Val 5 Thr	H: 13 amin DGY: YPE: ESCR: Ser Val	35 at no ac line prof	mino cid ear tein ON: Glu Pro Thr 40	SEQ Leu Tyr 25	ID NO Ala 10 Ala Val	O: 4 Phe Val Phe	Arg	Ile Leu Arg 45	Pro Thr 30	Val 15 Phe Leu	Glu		
1 Leu Leu	(ii) (xi) Ser Arg	(A) (II) (II) (II) (II) (II) (II) (II) (A) Li B) TO D) TO LECUI LEU Leu Glu 20	ENGTI YPE: DPOLO LE T' CE DI Val 5 Thr	H: 1: amin DGY: YPE: YPE: ESCR: Ser Val	35 at no ac line prof	mino cid ear tein ON: Glu Pro Thr 40	SEQ : Leu Tyr 25	ID NO Ala 10 Ala Val	O: 4 Phe Val Phe	Arg	Ile Leu Arg 45	Pro Thr 30	Val 15 Phe Leu	Glu		
1 Leu Leu Val	(ii) (xi) Ser Arg Pro Glu 50	(I)	A) Li B) TO C) TO LECUI QUENC Leu Glu 20 Asp Val	ENGTHYPE: DPOLO LE TY CE DI Val 5 Thr Ala	H: 1: amin DGY: YPE: ESCR: Ser Val Pro Asp	35 am no ac line pro Glu Asp Val Ala 55	mino cid ear tein ON: Glu Pro Thr 40 Ala	SEQ Leu Tyr 25 Trp Gly	ID No Ala 10 Ala Val	O: 4 Phe Val Phe Gly	Arg Arg Gly Asp 60	Ile Leu Arg 45 Val	Pro Thr 30 Glu Arg	Val 15 Phe Leu Val	Glu His Leu		

i,

						:::		x 37.	. s		٠.							
Phe	e Leu	Asp	Arg	Thr											Arg	Ala		
			100					10						10	_			
4																		
His	Ala	Asp 115		Asp	Ser	His	120		Asp	Al c	a ·Le		sn A 25	rg	Ser	Leu	•	•
Ala	Glu	Glu	Gln	Ser	Alá	Glý	Air Fi						:::	. ,	٠		· .	
	130	ş ·	fig. •		, i - , i"	¥135	(A, er	1. Y.			g å s	** . 1		nia ∂3	í-		T:	
÷ .																. 93		
(2)	INF											•					. :	
1-	(i) SE	OHEN	CE C	нара	ריי איני	TSTI	res .	:			.A.	/* s.	1,641	. ,			
	,-,	(A) L	ENGT	H: 4	d₁ 8′0	aše	pair	's		111 1	U_{ℓ}	1.50	713.74	. j.::	3 7	193	
			B) T C) S								٠.							
1		(D) T	OPOL	OGY:	lin	ear						1.6				100	. :
	(ii)) MO	LECU	LE T	YPE:	DNA	(ge	nomi	c)									
	(iii)) ну	POTH	ETIC	AL:	NO												
	(vi	OR	IGIN	AT, S	OTTRC'	R.												
		(.	A) 01	RGAN	ISM:	Str			s go	ldir	niens	sis	* 5 -	, "c 3%	. uv,	iji Mi		,
		(:	B) S'	rai:	N: A'	rcc :	2138	6							43.5	18 July 18		
	(ix)	.सच	ATURI	₹:							* * .							
	,,		A) N		KEY:	CDS												
			B) L	•								•	.,	. :	•			
		(1	D) 0:															
				/g	ene=	"ននឲ្	gA"			7.		`.		•	٠. * .	11.113		
	(xi)	SE	QUENC	CE D	ESCR:	IPTIC	ON: □	SEQ	ID N	0:5	: -				٠	,	2+	
ATG	AGC	TTC	CTC	GTC	TCG	GAA	GAA	CTC	TCC	TTC	CGI	' AT	т сс	2G - C	TG	GAG		48
	Ser																	
1				5					10						15			
ama	aam	m> «								•		•						
	CGT Arg																	96
DC u	m g	-7-	20		Cys			25		vai	: ALC		u 11			HIS		
										.,						7,		
	CCC																	144
neu	Pro	35	.wsp	ura	FIO	val	40	· rrp	WIG	rne	·· GT	4	_	.u L	eu l	Leu		
_	GAC																	192
Ile	Asp 50	Gly	Gly	Pro.	Arg	Pro 55	Cys	Gly	Asp	Gly	Asp 60		l Hi	s I,	le i	Ala		

Pro 65	Ala								•		;Ile		Leu	Gln	GTC Val 80		240
											GCG	:CCG	CTG	GTG			
									Pro		Gly	GJ.n	Glu 110			15. S	336
									GAG Glu	GCG Ala	CTG Leu	GAC Asp	CGC Arg	ATC Ile	CTG Leu		384
					GCC Ala				8: <u>1</u> -		/f:	ur s	0-1-1	j) 4	,,,;1	·*	408
(2)	INF	ORMAT	rion	FOR	SEQ					. \$. 15	art .	,	VIN Vija		19	((7)	
		(1	SEQUE	ENCE ENGTI	CHAR	ACTE	ERIST	rics acid	: ls			, ,				(mag)	
		(I) TC	POLO		line	ar		* #.								
		I) MOI	ECUI	POLO	OGY:	line	ear cein			r di	i ivii. Vii			į.		1 - 1	
	(xi) Ser	(I MOI SEÇ	ECUI ECUI UENC Leu	POLO LE TY LE DE	OGY: TPE: ESCRI Ser	line prot PTIC	ear Cein ON: S	EQ l	ID NO): 6:	Arg.	Ile	Pro	Val	Glu		
1 Leu	(xi) Ser Arg	(I MOI SEC Phe Tyr	D) TO LECUI QUENC Leu Glu 20	DPOLO LE TY LE DE Val 5	OGY: (PE: SSCRI Ser Cys	line prot PTIC Glu Asp	ear cein ON: S Glu Pro	EQ Leu Tyr 25	Ser 10): 6: Phe	Arga Arga Arga Arg	Ile Leu	Pro Thr	Val 15 Phe	Glu His		
1 Leu	(xi) Ser Arg	MOI SEC Phe Tyr Gly 35	ECUI QUENC Leu Glu 20	DPOLO LE TY LE DE Val 5 Thr	OGY: OFF: SECRI Ser Cys Pro	line prot PTIC Glu Asp	ear cein DN: S Glu Pro Thr 40	Leu Tyr 25	Ser 10 Ala Ala	Pherodal Val	Arg Arg Gly	Ile Leu Arg 45	Pro Thr 30	Val 15 Phe	Glu His Leu		Program A 1410 Maria
1 Leu Leu	(xi) Ser Arg	MOI SEC Phe Tyr Gly 35	D) TO LECUI QUENC Leu Glu 20	DPOLO DE TY DE DE Val 5 Thr Ala	OGY: (PE: SCRI Ser Cys	line prot PTIC Glu Asp Val	ear cein ON: S Glu Pro Thr 40	EQ 1 Leu Tyr 25 Trp	Ser 10 Ala Ala	Phe Val	Arg Arg Gly	Ile Leu Arg 45	Pro Thr 30	Val 15 Phe	Glu His Leu	e e e e e e e e e e e e e e e e e e e	**************************************
1 Leu Leu Ile	(xi) Ser Arg Pro Asp 50	MOI SEC Phe Tyr Gly 35 Gly	ECUI QUENC Leu Glu 20 Asp Gly	DPOLO LE TY LE DE Val 5 Thr Ala Pro Glu	OGY: (PE: SCRI Ser Cys Pro Arg	line prot PTIC Glu Asp Val Pro 55	cein ON: S Glu Pro Thr 40 Cys	Leu Tyr 25 Trp Gly	Ser 10 Ala Ala Asp	Phe Val Phe	Arg Gly Asp 60	Ile Leu Arg 45 Val	Pro Thr 30 Glu His	Val 15 Phe Leu Ile	Glu His Leu Ala Val		Party Control of the
Leu Leu Ile Pro 65	(xi) Ser Arg Pro Asp 50 Ala	MOI SEC Phe Tyr Gly 35 Gly	ECUI QUENC Leu Glu 20 Asp Gly	DPOLO LE TY Val S Thr Ala Pro	OGY: (PE: SSCRI Ser Cys Pro Arg Thr 70	line prot PTIC Glu Asp Val Pro 55 Phe	cein ON: S Glu Pro Thr 40 Cys Gly Arg	Leu Tyr 25 Trp Gly Glu Val	Ser 10 Ala Ala Val Gly 90	Phe Val	Arg Gly Asp 60 Ile	Ile Leu Arg 45 Val Arg	Pro Thr 30 Glu His	Val 15 Phe Leu Ile Gln Val 95	Glu His Leu Ala Val 80		Party Comments of the Comments

Leu	Ala	Asp Phe	Asp Al	a Leù	Leu A	sp Glu	ı Ala	Leu	Asp Ar 125	g Ile	e. Leu		
Ala	Glu	Glu Gln	Asn Al	a Gly									
•	130												
		J. 1 1	1 2 50	ST 3.1.			* 17.4	4.00	26.51			:	
		113			i.	1 a _				1-2			
7			5										:
(2)	INF	ORMATION	FOR SE	Q ID 1	10: 7:				. Stylle is	rdi y	** 5		
	/21	anoim.	an <i>a</i>	3	- am- aa					4	٠.		
2.	(1)	SEQUEN	CE CHAR ENGTH:					12 m		5528 85			
			YPE: nu										
			TRANDED									٠,	
			OPOLOGY			•					**		
		(-, -,	02001				• .	200	. NE3 (1.5			
	(ii)	MOLECU	LE TYPE	: DNA	(genon	nic)							
	, ,											i i	
	(iii)	нүротні	ETICAL:	NO									
	(vi)	ORIGINA											
			RGANISM										
		(B) S'	TRAIN:	ATCC 2	23940		- :-		. '-1 3	-t '. · · ·	l'int	4 7	
	(i ~)	FEATURI	. ·					21.58		de la Colo	and the second		
	(12)		AME/KEY	· CDS									
			CATION						11 W				
			THER IN										
				= "ssg		-		_					
		•					€.	5	J. V		1.11	4	
	(xi)	SEQUENC	CE DESC	RIPTIC	N: SEÇ	D N	0: 7:		1	1.53	· ·	s. 5%	
አጥር!	NGC	TTC CTC	GTC TC	a ana	en ver lên	ים יוויםם	्रकेम्प्ल ः	አአረር. አ	ישמ. ממ	a (1997)	CNN		48
		Phe Leu											* 40
1		riic neu	5	2 014	GIU IIC	10	FILE	uys 1	TC FT.	15	GIU		
_			s. Širbi.	يى .	A SW		· .	. 3°					
CTG	CGA	TAC GAG									CAC		96
Leu	Arg	Tyr Glu	Thr Ar	g Asp	Pro Ty	r Ala	Val .	Arg M	let Th	r Phe	His		
	_		al of the	I	1) 1:75	_ 1 1 .		_		^			
		20				5	·	:	3	U.			
		20				5	ra di r Majo		: 3	U.			
CTC	CCC	GGA GAC					ψŞ				CTG		144
			GCG CC	T GTG o Val	ACC TG	G GCG	TTC :	GGC C	GG GA	G CTG			144
		GGA GAC	GCG CC	T GTG o Val	ACC TG	G GCG	TTC :	GGC C	GG GA	G CTG		*.	144
Leu	Pro	GGA GAC Gly Asp 35	GCG CC Ala Pr	T GTG o Val	ACC TG Thr Tr 40	G GCG p Ala	TTC Phe	GGC C	CGG GA Arg Gl	G CTG u Leu	. Leu 🖟	* . * . * 3*	
Leu	Pro	GGA GAC Gly Asp 35 GGG ATC	GCG CC Ala Pr	T GTG o Val	ACC TO Thr Tr 40	G GCG P Ala	TTC Phe	GGC C	CGG GA Arg Gl 45	G CTG u Leu C ATC	Leu:		144
Leu	Pro GAC Asp	GGA GAC Gly Asp 35	GCG CC Ala Pr	T GTG o Val C CCG	ACC TO Thr Tr 40	G GCG P Ala	TTC Phe	GGC C Gly A GAC G Asp V	CGG GA Arg Gl 45	G CTG u Leu C ATC	Leu:		
Leu	Pro	GGA GAC Gly Asp 35 GGG ATC	GCG CC Ala Pr AAC CG Asn Ar	T GTG o Val C CCG g Pro 55	ACC TG Thri Tr 40 AGC GG Ser Gl	G GCG P Ala	TTC Phe	GGC C	CGG GA Arg Gl 45	G CTG u Leu C ATC s Ile	GCC:		
Leu CTC Leu	Pro GAC Asp 50	GGA GAC Gly Asp 35 GGG ATC Gly Ile	GCG CC Ala Pr AAC CG Asn Ar	T GTG o Val C CCG g Pro 55	ACC TG Thri Tr 40 AGC GG Ser Gl	G GCG p Ala C GAC y Asp	TTC Phe GGC Gly	GGC C Gly A GAC G Asp V	CGG GA Arg Gl 45 GTC CA Val Hi	G CTG u Leu C ATC s Ile	GCC:		192
CTC Leu CCG	GAC Asp 50	GGA GAC Gly Asp 35 GGG ATC Gly Ile GAC CCC	GCG CC Ala Pr AAC CG Asn Ar	T GTG o Val C CCG g Pro 55	ACC TG Thri Tr 40 AGC GG Ser Gl	G GCG P Ala C GAC Y Asp	TTC Phe	GGC CGLy AGAC GAC GAC GAC GAC GAC GAC GAC GAC GA	CGG GAARG GI 45 GTC CAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	G CTG u Leu C ATC s Ile	GCC Ala		192
CTC Leu CCG	GAC Asp 50	GGA GAC Gly Asp 35 GGG ATC Gly Ile	GCG CC Ala Pr AAC CG Asn Ar	T GTG O Val C CCG G Pro 55 C CTG Y Leu	ACC TG Thri Tr 40 AGC GG Ser Gl	G GCG p Ala GC GAC y Asp	TTC Phe	GGC CGLy AGAC GAC GAC GAC GAC GAC GAC GAC GAC GA	CGG GAARG GI 45 GTC CAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	G CTG u Leu C ATC s Ile	GCC Ala		192

															Ala		288
								GTG Val 105				Gln					336
								GAG Glu	Ala	Ala		Gly 125	Lys				384
			CAG Gln				TG		. e .* _ :	11	ia (1) assati ottas	: :	i . "				408
<i>(</i> -)									۳.,.		. I THE					(14)	
(2)		(i) 5 (<i>I</i> (E	FION SEQUE A) LE B) T'S	ENCE ENGTI PE :	CHAI I: 13 amir	RACTI 35 ar	ERIST mino cid		: ls								
	(ii)	·	LECUI							'5' :	ċ	i a	k si		ş · .	147 - 25 2 - 2 - 3 - 3 - 3	
								SEQ 1	3 3	1.					: ". ; !	<i>3</i> .	
Met 1	Ser	Phe	Leu	Val 5	Ser	Glu	Glu	Leu	Ser 10		Lys	Ile	Pro	Val 15	Glu		
Leu	Arg	Tyr	Glu 20	Thr	Arg	Asp	Pro	Tyr 25			Arg		•		His	i i i	
Leu	Pro	Gly 35	Asp	Ala	Pro	Val	Thr 40	Trp	Ala	Phe	Gly	Arg 45	Glu	Leu	Leu	2.	
Leu	Asp 50	Gly	Ile	Asn	Arg	Pro 55	Ser	Gly			Asp 60		His	Ile	Ala		si si
Pro 65	Thr	Asp	Pro	Glu	Gly 70	Leu	Ser	Asp	Val	Ser 75	Ile	Arg	Leu	Gln	Val 80		
Gly	Ala	Asp	Arg	Ala 85	Leu	Phe	Arg	Ala	Gly 90		Pro		Leu	Val 95	Ala	· ·	1.13
Phe	Leu	Asp	Arg 100	Thr	Asp	Lys	Ser	Val 105	Pro	Leu		Gln	Glu 110	Gln	Thr		
Leu	Gly	Asp 115	Phe	Glu	Asp	Ser	Leu 120	Glu	Ala _.	Ala	Leu	125	Lys	Ile	Leu		

.

:

Ala	Glu (Glu Gln	Asn Ala	.Gly ;; ∈ 135	. 126 13	31.3	*1	1					. * *	٠.
	130			200	¥									
: •						157								n
(2)	INFO	RMATION	FOR SEQ	ID NO: 9		*** ,£								
	(i)	SEQUENC (A) LE (B) TY	E CHARAC NGTH: 34 PE: nucl	TERISTIC h base pa leic acid	s: irs	. 1 =		\$ ¹ 2	1.j. 1	1.00	**:			
14			RANDEDNE POLOGY:	SSS: sing linear	le	•	. 17				137 237			
	(ii)	MOLECUL	E TYPE:	DNA (syn	theti	Lc)		i					in a	
	(vi)	ORIGINA (C) IN		E: L ISOLATE	: 589	, 31								
	(xi)	SEQUENC	E DESCRI	PTION: S	EQ II		9:						3 .	
GGC	GAATT(CG AACAG	CTACG TO	egcgaagtc	GCC2	A	:) . : . :						34
(2)	INFO	RMATION	FOR SEQ	ID NO: 1	0:			pris.	· :	22.5	:		: .	•
	(i)	(A) LE (B) TY (C) ST	NGTH: 32	CTERISTIC base pa leic acid ESS: sing linear	irs						. (%1) (. (%)		1 + 1 + 1 + 1	
	(ii)	MOLECUL	E TYPE:	DNA (syn	thet:	ic)	**	4.5			r e	÷()	\	. *
	(vi)	ORIGINA (C) IN	L SOURCE	E: ATC LATE L ISOLATE	: ss	3 2	9.7.		4					1 1 0
				IPTION: , S		D NO:	: 10:	: -	in p	: *		1		
GTG	GGATC			CTGGTCGTC										32
			• 190	7. 3.11 Tue 23	<i>f.</i>		•		<i>r</i>		J			
(2)		•		ID NO: 1		•	· · ·	:	, .	\$;	6,1.0	*		;
		(A) LE (B) TY (C) ST (D) TO	NGTH: 3 PE: nuc RANDEDN POLOGY:	CTERISTIC 2 base pa leic acid ESS: sing linear	irs l le	:		•	e eu			gradia G		
	(ii)	MOLECUI	E TYPE:	DNA (syn	ithet	lC)								

- (vi) ORIGINAL SOURCE:
 - (C) INDIVIDUAL ISOLATE: ssg3
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

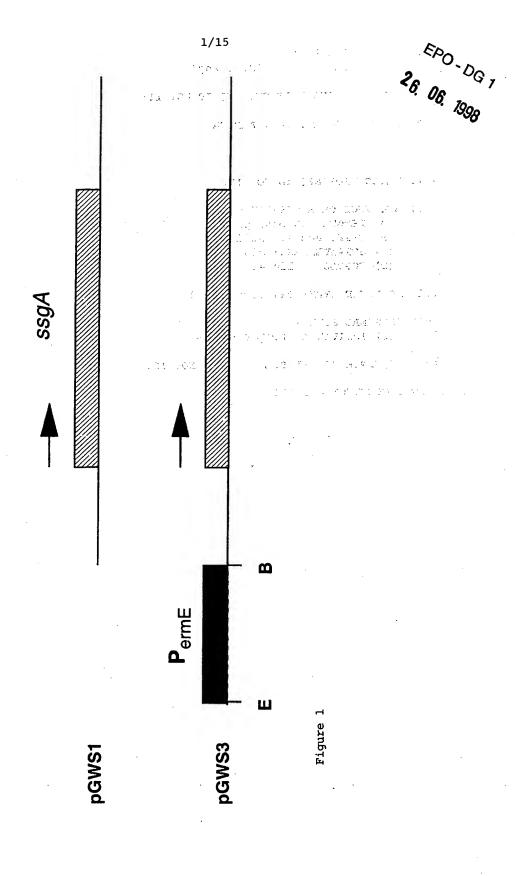
GGGAATTCCA TATGCGCGAG TCGGTTCAAG CA

32

- (2) INFORMATION FOR SEQ ID NO: 12:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid;
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: DNA (synthetic)
 - (vi) ORIGINAL SOURCE:
 - (C) INDIVIDUAL ISOLATE: ssg4
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CCGGTCAGCC GGCGTTCTGC TCCTC

25



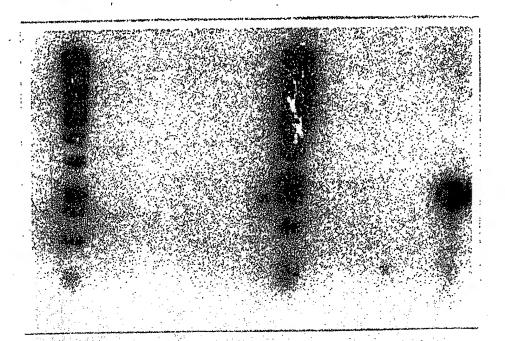


Figure 2A

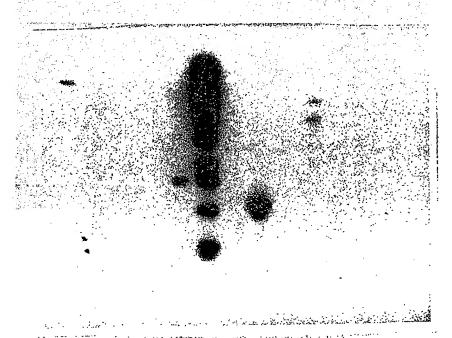


Figure 2B

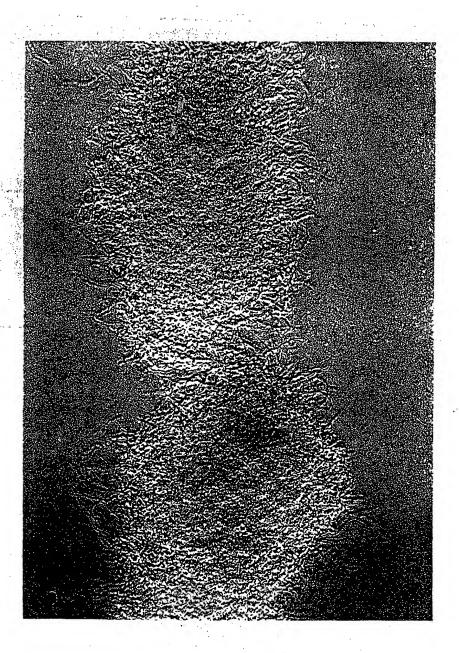
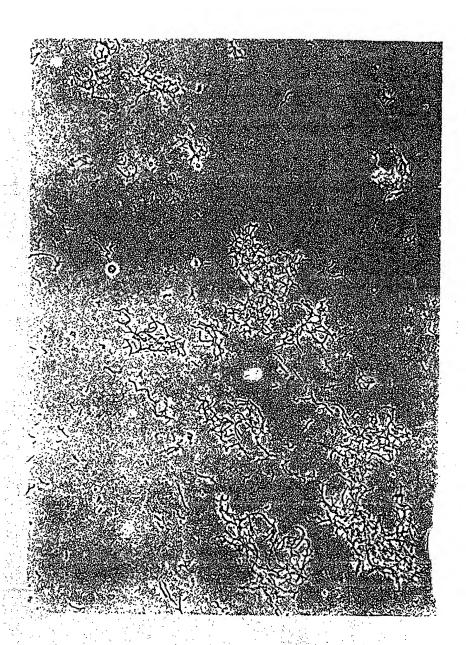
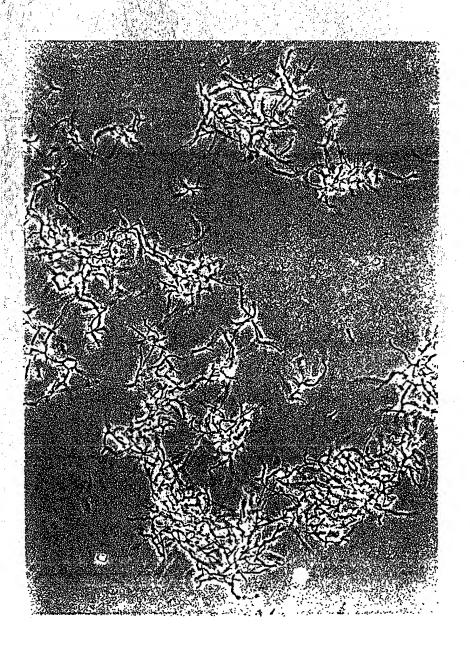


Figure 3A





SEQUENCE LISTING

	•	SEQUENCE DISTIN			
		The Control of the Control		F - 1 17 17 12 1	
(1) GENE	ERAL INFORMATION:	** *		•	
· (i)	APPLICANT:		··· · · · · · · · · · · · · · · · · ·	:	
	(A) NAME: Rijksunive	rsiteit Leiden		A 1 - 112	
	(B) STREET: Wassenaar				
		rseweg /2			
	(C) CITY: Leiden	and the second second			
	(E) COUNTRY: The Neth	herlands		1.1	**
	(F) POSTAL CODE (ZIP)): 2300 RA		100	1
			* ;		
(ii)	TITLE OF INVENTION: Re	educing branching	and enha	ncing	
,,	fragmentation in cu	ulturing filamen	tous micro	organisms	() .
				5 and	1 8
				to the .	
(111)	NUMBER OF SEQUENCES: 1	12 -			
	/				
(iv)	COMPUTER READABLE FORM	MI: A ATT OF THE TOTAL TO THE FO	satural delication		
	(A) MEDIUM TYPE: Flor	opy disk			600 - 100
	(B) COMPUTER: IBM PC			, 7	
	(C) OPERATING SYSTEM:				
			***************************************	/=	
	(D) SOFTWARE: Patentl	In Release #1.0,	version #	1.25 (EPO	,
					The second
	: ";	657		£. Ç	•
(2) INFO	RMATION FOR SEQ ID NO:	1:			
		H	en end the	4 4 4	f 42 .
(2)	SEQUENCE CHARACTERIST		The state of		112
(1)		:			
	(A) LENGTH: 408 base				5.4
	(B) TYPE: nucleic aci	id			
	(C) STRANDEDNESS: dou	uble			
	(D) TOPOLOGY: linear				
	(-,				
(ii)	MOLECULE TYPE: DNA (ge	enomic)	or en la gra	17 . 77 × 1	100
(iii)	HYPOTHETICAL: NO	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	. 1 11 111 1111	1 12	
		12.25 5444	1. 34		
(ari)	ORIGINAL SOURCE:	1	4. 1	din jer	
(41)	(A) ORGANISM: Strepto				•
	(A) ORGANISM: Strepto	omyces griseus		. •	
	(B) STRAIN: ATTC 2334	15			
	•	1.0 9/18/20	7 34 7		-:
(ix)	FEATURE:				
	(A) NAME/KEY: CDS	- 70 <u></u>	A 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2		13 A
	(B) LOCATION: 1408				
	(D) OTHER INFORMATION		~ n	: : :	
		a: /product- say	355	:	
	/gene= "ssgA"			•	
(xi)	SEQUENCE DESCRIPTION:	SEQ ID NO: 1:		•	27.44
	.:	. ;		•	
ATG AGC	TTC CTC GTC TCC GAG GAG	G CTC TCG TTC CG	r att ccg	GTG GAG	41
	Phe Leu Val Ser Glu Glu				* *
	5	10	, === ===	15	•
1)	TO		±.0	

CTC CGA TAC GAG GTC GGC GAT CCG TAT GCC ATC CGG ATG ACG TTC CAC

Leu Arg Tyr Glu Val Gly Asp Pro Tyr Ala Ile Arg Met Thr Phe His

25

96

30

20

															CTG		144
Leu	Pro	Gly 35	Asp	Ala	Pro	Val	Thr 40	Trp	Ala	Phe	Gly	Arg 45		Leu	Leu	٠	s.
CTG	GAC	GGG	CTC	AAC	AGC	CCG	AGC	GGC	GAC	GGC	GAT	GTG	CAC	ATC	GGC		192
															Gly		
	50					55		ν,	112	3, 50	- 60	•					
CCG	ACC	CAG	רככ	GAG	GGC.	ርጥሮ	GGA	GAT	· GTC	CAC	- አጥር		יי. מיזיט		(200°C)		240
							Gly										240
65					70		_	_	_	75					80		
a aa	000	~~~													3.7	N = 4	
							CGG Arg										288
1			3	85											- 5	٠,.	
TTC	CTC	GAC	CGG	ACG	GAC	AAG	CTC	GTG	CCG	CTC	GGC;	CAG	GAG	CAC	ACG	9.3	336
Phe	Leu	Asp		Thr	Asp	Lys	Leu									•••	
			100										110				
CTG	GGT	GAC	TTC	GAC	GGC		CTG										384
							Leu										-
		115					120					125					
GCC	GAG	GAG	CAG	AAC	GCC	GGC	TG			1)	i, į		Mar I	4	`	1,1	408
			Gln						; :	;	·					f.s	
	130					135							٠ ٤, ٠				
													10 to		. Sa "		
									•							•	
(2)	TATELO	\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	17017	50D	CEO	TD 1	TO 6										
(2)	INFC)KIMA	LON	FOR	SEQ	ID I		4: .					: 1		e 5.°.		•
	(RIST						1 1	r. : . i '			
			A) LE B) TY				nino	acid	is						, .		
							ar _{ij}		· · ·	. ;							
		•													٠, '		
	(ii)	MOI	ECUI	E TY	PE:	prot	ein										
	(xi)	SEC	UENC	E DE	SCRI	PTIC	N: S	EQ I	D NC						. : . : *	.*	
Met	Ser	Phe	Leu	Val	Ser	Glu:	Glu	Leu	Ser	Phe	arg.	Ile		Val	Glu		
1				5					10				nage .	15	•		
Leu	Ara	ጥህ <u>ነ</u> ት	Glu	Val	Glv	Δsn	Pro	ጥህጉ	Δla	Tle.	Δrσ	Met.	Thr	Dhe	His		
	3	-1-	20					25			·		30	*****			
Len	Pro	៨1 v					Thr				g) v	Δτα	Glu	T.e.i	Leu	79. at 1. s	
- u		35	P	· sré de	~~0.	* (4.4.	40		A. C.	a 44C	JIY	45	Jau	4e a	Lieu.	1,74	
Leu	Asp	Gly	Leu	Asn	Ser	Pro	Ser	Gly	Asp	Gly	Asp	Val	His	Ile	Gly		
	50	-				55	• •		-	-	60		•			-	

Pro 65	Thr	Glu 1	Pro Gli	1 Gly 70	Leu Gly		Val Hi	Ę.,	٠.	* *		Val 80		
Gly	Ala	Asp i	Arg Ala	a Leu	Phe Arg	Ala	Gly Th	r Ála	Pro	Leu	Val	Ala		
. ,		٠.	8.	5			90 	04 1.			95			
Phe	Leu	_	Arg Th		Lys Leu				Gln	Glu 110	His	Thr	•	
Leu	Gly	Asp 1	Phe Asj	Gly	Asn Leu 120	Glu	Asp Aid	Leu	Gly 125	Arg	Ile	Leu	, , 1,	·. •
Ala	Glu 130	Glu (n Ala An Nga ∫C Nga An	Gly 135	1. 1 93 81 331	1 71: D 8 11: 1 8 11:	90 (M 16 (F)	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	 		n ngang	٠.	
(2)	INFO				ID NO:		44.3		4 -2 -3	; .				
	(i)	(A) (B) (C)	LENGT TYPE STRAI	TH: 40 : nucl NDEDNE	TERISTI 8 base eic aci SS: dou linear	pairs d						- 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1		
			CULE C		DNA (ge	nomic)			:	; •	7 **		: 3
	(vi)	(A)		NISM:	: Strepto CC 3004	myces	albus	G iff		7.	· • •			
٠.	(ix)		NAME,		CDS 1408							AFLI.	. 4 / /	
		(D)			RMATION	: /pro	oduct=	"Ssg/	/ n	. 441	d. (1984)	F 3	· · ·	
	(xi)				"ssgA"		D NO: 3		100		· .	•	y=	`a.*
		Phe I	Leu Val	Ser	GAG GAG Glu Glu	Leu I	4 60	arg					٠.	48
CTG	CGG				GAT CCG				CTG	ACG	TTC	CAC	•	96
		Tyr (val	Asp Pro		Ala Val		Leu	Thr	Phe	His	·.	
					GTC ACC									144
Leu	Pro	Gly 2 35	Asp Ala	Pro	Val Thr 40	. –	Val Phe	e Gly	Arg 45	GLu	Leu	Leu		

GTC Val	GAG Glu 50	GGA Gly	Val	Leu	GAC Asp	Ala	Ala	Gly	Asp	Gly	Asp	Val	CGG	GTC Val	TGC Cys		192
CCG Pro 65	GTG	GGG Gly	CAG	ACG	GCC Ala 70	ACC	AGG	GAG	GTG Val	CAC	ATC	ACC	CTC Leu	CAG Gln	GTC Val 80		240
GGC Gly	TCC Ser	GAG Glu	CAG Gln	GCG Ala 85	CTC Leu	TTC Phe	CGO Arg	GTC Val	GGC Gly 90	AAG Lys	GCG Ala	CCG Pro	CTG Leu	CTC Leu 95	GCC Ala		288
TTC Phe	CTC Leu	GAC Asp	CGC Arg 100	ACC Thr	GAC Asp	CAG Gln	GGC Gly	TTG Leu 105	TCG Ser	CTC Leu	GGC Gly	AGC Ser	GAG Glu 110	CGG Arg	GCA Ala		336
					AGC Ser				Asp	Ala	Leu	Asn 125	Arg	Ser	Leu	, * t *	384
					GCC Ala					e		entina Birlina	7.6 3 + 3 4 (4)	etti ja Linuari Linuari	\$	``	408
(2)		(i) S	EQUI	ENCE	SEQ CHAF	RACTI	ERIST	rics		•			慢		g eta Santua Na Pa	1	
		(E	3) T	PE:	H: 13 amir DGY:	10 a	cid	acir	15						 		
					YPE:	_			ID NO): 4:	j# a2 4 s •a + s +			1000 14 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		.1 .7 1	
Met 1	,		-		Ser		Glu	Leu	Ala	Phe	Arg	Ile	Pro	Val		· ; · · ·	
Leu															His	91 	
Leu	Pro	Gly 35	Asp	Ala	Pro	J. 1	40			Phe	Gly	Arg 45					
Val	Glu 50	Gly	Val		Asp	55	Ala	Gly	Asp	Gly	60	Val	Arg	Val	Суз		
Pro 65	Val	Gly		Thr		Thr			Val			Thr	Leu	Gln	Val 80	: .	,
Gly	Ser	Glu	Gln	Ala	Leu	Phe	Arg	Val	Gly	Lys	Ala	Pro	Leu	Leu			

	· 9,0 , · · · · · · · · · ·	95, 543	1 1
Phe Leu Asp Arg Thr Asp Gin Gly Let 100 109	i Ser Leu Gly S	er Glu Arg Ala 110	
His Ala Asp Phe Asp Ser His Leu Asp 115 120	Asp Ala Leu A	sn Arg Ser Leu	4. 1% 163
Ala Glu Glu Gln Ser Ala Gly The Late 130 The Control of the Contro	THE OBSTACE Flux SEE SAME CO	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	man is nem or h
(2) INFORMATION FOR SEQ ID NO: 5:	the got had ned		
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 408 base pair (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear		DIR Brown Libert and Brown Drog a A	
(ii) MOLECULE TYPE: DNA (genomi		and the state of the season	
(iii) HYPOTHETICAL: NO			
<pre>(vi) ORIGINAL SOURCE: (A) ORGANISM: Streptomyce (B) STRAIN: ATCC 21386</pre>	s goldiniensis	 A. Carlotto, and the control of the co	
(ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 1408 (D) OTHER INFORMATION: /p	roduct= "SsgA"		* *
/gene= "ssgA"	Casa ste Lo	and and such extra	228 John
(xi) SEQUENCE DESCRIPTION: SEQ			-
ATG AGC TTC CTC GTC TCG GAA GAA CTC Met Ser Phe Leu Val Ser Glu Glu Leu	Ser Phe Arg I	le Pro Val Glu	
$1 = 1 = \{1, 2, 3, 3, 5, \mathbf$	10 ₂₂ - 1 ₁₁	1.5	·
CTG CGT TAC GAG ACC TGT GAT CCC TAC Leu Arg Tyr Glu Thr Cys Asp Pro Tyr 20 25	Ala Val Arg Le		96
CTG CCC GGA GAT GCC CCG GTG ACC TGG Leu Pro Gly Asp Ala Pro Val Thr Trp 35	Ala Phe Gly A	and the second s	144
ATC GAC GGA GGT CCG CGG CCG TGC GGG Ile Asp Gly Gly Pro Arg Pro Cys Gly		5 4	192

GGG AGC GAG CAG GAG ATG TTC CGG GTC GGC ACG GCG CTG GTG GCC Gly Ser Asp Gln Ala Met Phe Arg Val Gly Thr Ala Pro Leu Val Ala 85 TTC CTG GAC CGC ACG GAC AAG ATC GTG CCG CTG GGG CAG GAG CGT TCC Phe Leu Asp Arg Thr Asp Lys Ile Val Pro Leu Gly Gln Glu Arg Ser 100 CTC GCC GAC TTC GAC GCC CTG CTC GTG CAG GAG GCG CTG GAC CGC ATC CTG Leu Ala Asp Phe Asp Ala Leu Leu Asp Glu Ala Leu Asp Arg Ile Leu 115 GCC GAG GAG CAG AAC GCC GGC TG Ala Glu Gln Gln Asn Ala Gly 130 (2) INFORMATION FOR SEQ ID NO: 6: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 135 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6: Met Ser Phe Leu Val Ser Glu Leu Ser Phe Arg Ile Pro Val Glu 1 5 10 Leu Arg Tyr Glu Thr Cys Asp Pro Tyr Ala Val Arg Leu Thr Phe His 20 20 Leu Pro Gly Asp Ala Pro Val Thr Trp Ala Phe Gly Arg Glu Leu Leu 35 40 40 45 Cle App Gly Gly Pro Arg Pro Cys Gly Asp Gly Asp Val His Ile Ala 50 Pro Ala Asp Pro Glu Thr Phe Gly Glu Val Leu Ile Arg Leu Gln Val 65 70 75 80 Cly Ser Asp Gln Ala Met Phe Arg Val Gly Thr Ala Pro Leu Val Ala 85 Phe Leu Asp Arg Thr Asp Lys Ile Val Pro Leu Gly Gln Glu Arg Ser 100 Phe Leu Asp Arg Thr Asp Lys Ile Val Pro Leu Gly Gln Glu Arg Ser 100		Ala	Asp	Pro	Glu	Thr	Phe	Gly	Glu	Val		Ile	Arg					240
Phe Leu Asp Arg Thr Asp Lys Ile Val Pro Leu Gly Gln Glu Arg Ser 100 105 105 384 CTC GCC GAC TTC GAC GCC CTG CTC GAC GAG GCG CTG GAC CGC ATC CTG Asp Phe Asp Ala Leu Leu Asp Glu Ala Leu Asp Arg Ile Leu 115 120 125 207 306 384 GCC GAG GAG CAG AAC GCC GGC TG Ala Glu Glu Glu Glu Gln Asn Ala Gly 135 207 307 307 307 307 307 307 307 307 307 3					Ala					Gly	Thr				Val	Ala		
Leu Ala Asp Phe Asp Ala Leu Leu Asp Glu Ala Leu Asp Arg Ile Leu 115				Arg					Val					Glu			• • •	336
Ala Glu Glu Gln Asn Ala Gly 130 135 (2) INFORMATION FOR SEQ ID NO: 6: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 135 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6: Met Ser Phe Leu Val Ser Glu Glu Leu Ser Phe Arg Ile-Pro Val Glu 1 1 5 10 15 Leu Arg Tyr Glu Thr Cys Asp Pro Tyr Ala Val Arg Leu Thr Phe His 20 25 30 Leu Pro Gly Asp Ala Pro Val Thr Trp Ala Phe Gly Arg Glu Leu Leu 35 40 45 Tle Asp Gly Gly Pro Arg Pro Cys Gly Asp Gly Asp Val His Ile Ala 50 Pro Ala Asp Pro Glu Thr Phe Gly Glu Val Leu Ile Arg Leu Gln Val 65 70 Gly Ser Asp Gln Ala Met Phe Arg Val Gly Thr Ala Pro Leu Val Ala 85 90 Phe Leu Asp Arg Thr Asp Lys Ile Val Pro Leu Gly Glu Glu Arg Ser			Asp					Leu	Asp	Glu	Ala	Leu	Asp	Arq	Ile	Leu	efr' l	384
(2) INFORMATION FOR SEQ ID NO: 6: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 135 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6: Met Ser Phe Leu Val Ser Glu Glu Leu Ser Phe Arg Ile Pro Val Glu 1 5 10 15 Leu Arg Tyr Glu Thr Cys Asp Pro Tyr Ala Val Arg Leu Thr Phe His 20 25 30 Leu Pro Gly Asp Ala Pro Val Thr Trp Ala Phe Gly Arg Glu Leu Leu 35 40 45 Ile Asp Gly Gly Pro Arg Pro Cys Gly Asp Gly Asp Val His Ile Ala 50 55 60 Pro Ala Asp Pro Glu Thr Phe Gly Glu Val Leu Ile Arg Leu Gln Val 65 70 75 80 Gly Ser Asp Gln Ala Met Phe Arg Val Gly Thr Ala Pro Leu Val Ala 85 90 95 Phe Leu Asp Arg Thr Asp Lys Ile Val Pro Leu Gly Gln Glu Arg Ser		Glu					Gly			3	ere e. Pess	orad List	994 6000 6000	- AIS O - P Echer	MOA TOT SPEL	121 141 121	ă B	408
(B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6: Met Ser Phe Leu Val Ser Glu Glu Leu Ser Phe Arg Ile-Pro Val Glu 1 5 10 15 Leu Arg Tyr Glu Thr Cys Asp Pro Tyr Ala Val Arg Leu Thr Phe His 20 20 25 30 Leu Pro Gly Asp Ala Pro Val Thr Trp Ala Phe Gly Arg Glu Leu Leu 35 40 45 Tle Asp Gly Gly Pro Arg Pro Cys Gly Asp Gly Asp Val His Ile Ala 50 55 60 Pro Ala Asp Pro Glu Thr Phe Gly Glu Val Leu Ile Arg Leu Gln Val 65 70 75 80 Gly Ser Asp Gln Ala Met Phe Arg Val Gly Thr Ala Pro Leu Val Ala 85 90 95 Phe Leu Asp Arg Thr Asp Lys Ile Val Pro Leu Gly Gln Glu Arg Ser	(2)	INFO	ORMA'	rion	FOR	SEQ	ID 1	NO: 6	6:	. C a 1	fig (
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6: Met Ser Phe Leu Val Ser Glu Glu Leu Ser Phe Arg Ile Pro Val Glu 1 5 10 15 Leu Arg Tyr Glu Thr Cys Asp Pro Tyr Ala Val Arg Leu Thr Phe His 20 25 30 Leu Pro Gly Asp Ala Pro Val Thr Trp Ala Phe Gly Arg Glu Leu Leu 35 40 45 Ile Asp Gly Gly Pro Arg Pro Cys Gly Asp Gly Asp Val His Ile Ala 50 55 60 Pro Ala Asp Pro Glu Thr Phe Gly Glu Val Leu Ile Arg Leu Gln Val 65 70 75 80 Gly Ser Asp Gln Ala Met Phe Arg Val Gly Thr Ala Pro Leu Val Ala 85 90 95		(· a1 %		1,434,9	9 777.7		
Met Ser Phe Leu Val Ser Glu Glu Leu Ser Phe Arg Ile Pro Val Glu 1			(1 (1	A) LI B) TY O) TO	ENGTI (PE : (POL)	I: 13 amir DGY:	35 an no ac line	mino cid ear	acio	is	51) (1) 10	geori	1 10	erai Gera	CRO SYN	tvii	j, .	
Leu Pro Gly Asp Ala Pro Val Thr Trp Ala Phe Gly Arg Glu Leu Leu 35 40 45 Ile Asp Gly Gly Pro Arg Pro Cys Gly Asp Gly Asp Val His Ile Ala 50 55 60 Pro Ala Asp Pro Glu Thr Phe Gly Glu Val Leu Ile Arg Leu Gln Val 65 70 75 80 Gly Ser Asp Gln Ala Met Phe Arg Val Gly Thr Ala Pro Leu Val Ala 85 90 95 Phe Leu Asp Arg Thr Asp Lys Ile Val Pro Leu Gly Gln Glu Arg Ser		(ii)	() (1) (1) MOI	A) LI B) TY D) TO	ENGTH (PE: (POL(H: 13 amir DGY: VPE:	35 and a control of the control of t	mino cid ear cein	acio	is	D: 6	yead Daib		ং নার ১ বুল ১১ ক	080 248 : 820 : 100 10	(A) to i (A) (A)	j, .	
35 40 45 Ile Asp Gly Gly Pro Arg Pro Cys Gly Asp Gly Asp Val His Ile Ala 50 55 60 Pro Ala Asp Pro Glu Thr Phe Gly Glu Val Leu Ile Arg Leu Gln Val 65 70 75 80 Gly Ser Asp Gln Ala Met Phe Arg Val Gly Thr Ala Pro Leu Val Ala 85 90 95 Phe Leu Asp Arg Thr Asp Lys Ile Val Pro Leu Gly Gln Glu Arg Ser		(ii) (xi)	(I (I (I) MOI) SE(A) LECUI	ENGTH (PE: DPOLO LE TY CE DI Val	H: 13 amir OGY: VPE: ESCRI	35 am line prot	mino cid ear cein ON: S	SEQ 1	ID NO Ser 10	0: 6: Phe	year parth (TA- Arg	Ile	· · · · · · · · · · · · · · · · · · ·	0.40 0.40 0.40 0.40 0.40 0.40 Val	(/U) to:1 relative tide tide tide Glu	18 ·	
Pro Ala Asp Pro Glu Thr Phe Gly Glu Val Leu Ile Arg Leu Gln Val 65 70 75 80 Gly Ser Asp Gln Ala Met Phe Arg Val Gly Thr Ala Pro Leu Val Ala 85 90 95 Phe Leu Asp Arg Thr Asp Lys Ile Val Pro Leu Gly Gln Glu Arg Ser	1	(ii) (xi) Ser	(I (I (I) MOI) SEQ Phe	A) LH B) TY C) TO LECUI QUENC Leu Glu C20	ENGTH YPE: DPOLO LE TY CE DI Val 5	amir OGY: VPE: ESCRI Ser	35 and according to the second	mino cid car cein ON: S Glu Pro	SEQ 1	ID NO	Phe Val	year na na na na na na na na na na na na na na na na n	Île	Pro Thr	0.40 2000 1000 1000 1000 Val 15	(/C) the control of t	de la companya de la	
65 70 75 80 Gly Ser Asp Gln Ala Met Phe Arg Val Gly Thr Ala Pro Leu Val Ala 85 90 95 Phe Leu Asp Arg Thr Asp Lys Ile Val Pro Leu Gly Gln Glu Arg Ser	Leu Leu	(ii) (xi) Ser	(I (I) MOI) SEG Phe Tyr	LECUI LECUI Leu Glu Asp	ENGTH (PE: OPOLO LE TY CE DI Val 5 Thr	amir DGY: VPE: ESCRI Ser Cys	35 am lo ac line prot PTIC Glu Asp	mino cid cear cein ON: S Glu Pro C C C A etc	SEQ 1	ID NO	Phe Val	Gly	Ile Leu Arg 45	Pro Thr 30	CAN NY E SAN TO NY E Val 15 Phe Leu	(//) tell ferror for for for for for for for for for		
Phe Leu Asp Arg Thr Asp Lys Ile Val Pro Leu Gly Gln Glu Arg Ser	Leu	(ii) (xi) Ser Arg Pro	(I) (I) MOI SECONDARY Phe Tyr Gly 35	LECUI LECUI Leu Glu Asp	ENGTH (PE: (POLO) LE TY Val Val 5 Thr	amir DGY: VPE: SSCRI Ser Cys	S5 are and according to the second se	mino rid	EQ Tyr	ID NO Ser 10 Ala	Phe Val	Arg Gly	Ile Leu Arg 45	Pro Thr 30	OAN NYE SACTORY Val 15 Phe Leu	(//: field virial viria		
Phe Leu Asp Arg Thr Asp Lys Ile Val Pro Leu Gly Gln Glu Arg Ser	Leu Leu Leu Pro	(ii) (xi) Ser Arg Pro Asp 50	() (I) (I) (I) (I) (I) (I) (I) (I) (I) (LECUI LECUI Leu Glu Asp	ENGTH (PE: (POLC) LE TY CE DI Val 5 Thr Ala	Arg Thr	S5 ar no ac line prot CPTIC Glu Asp Val Prof 55	cin	SEQ Leu Tyr 25 Trp Gly	ID NO Ser 10 Ala Ala Asp	Phe Val Phe Gly Leu 75	Arg Arg Gly Asp 60	Ile Leu Arg 45 Val	Pro Thr 30 Glu His	Val Phe Leu	(//: in-) in-		1191
	Leu Leu Pro 65	(ii) (xi) Ser Arg Pro Asp 50 Ala	(A) MOI SEG Phe Tyr Gly 35 Gly	A) LH B) TY C) TO LECUI QUENC Leu Glu 20 Asp Gly Pro	ENGTH (PE: (POLC) (POLC	Arg Thr 70 Met	S ar	mino rid rein on: Second rein on: Second rein on: Second rein on rein	SEQ Leu Tyr 25 Trp Gly Glu	ID NO Ser 10 Ala Ala Asp Val	Phe Val Phe Gly Leu 75	Arg Arg Gly Asp 60 Ile	Ile Leu Arg 45 Val Arg	Pro Thr 30 Glu His	Val Fle Glin Val 95	(//: is-i is-i is-i is-i is-i is-i is-i is-		1191

115 120	p Giu Ala Leu Asp Arg Ile Leu
115. 120	(5) みん カルケット 125 ことが かん 大きた ルー・デー
Ala Glu Glu Gln Asn Ala Gly	·
130 135	o de la companya del companya de la companya del companya de la co
	kan terdah dalam terdah di kecamatan di Kebupaten dalam dalam berasakan dalam berasakan dalam berasakan dalam Kebupaten dalam berasakan dalam berasakan dalam berasakan dalam berasakan dalam berasakan dalam berasakan dalam
	ski dan dase ega oli elim degi (lagge elim)
	(文本) (
(2) INFORMATION FOR SEQ ID NO: 7:	
	74
(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 408 base pair	rs - Die Mike Liebergker in der gegen der
(B) TYPE: nucleic acid	Althoration 8
(C) STRANDEDNESS: double	24.4
(D) TOPOLOGY: linear	
(ii) MOI POIL P TUDE DE /)
(ii) MOLECULE TYPE: DNA (genomi	.c)
(iii) HYPOTHETICAL: NO	
(144) Holling Comb. No	化活动 经销售债金 计工作证据 医侧侧
(vi) ORIGINAL SOURCE:	na in kalamber kasa dengasa julya iye
	s, netropsis
(B) STRAIN: ATCC 23940	
·	
(ix) FEATURE:	•
(A) NAME/KEY: CDS	FELD CARDON BOOK AND A SECTION
(B) LOCATION: 1408	
(D) OTHER INFORMATION: :/p /gene= "ssqA"	roduct= "SsgA"
	v. v.
	to the total and the second of the second
(xi) SEQUENCE DESCRIPTION: SEQ	ID NO: 7.
A TO SME A SME OF A BEAUTIFUL TO	
ATG AGC TTC CTC GTC TCC GAG GAG CTC	TCC TTC AAG ATC CCA GTC GAA
Met Ser Phe Leu Val Ser Glu Glu Leu	Ser Phe Lys Ile Fro Val Glu
- 1 - 1,500	10 m Selection 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
t_{ℓ}	N.A.
IG CGA TAC GAG ACC CGG GAT CCC TAC	GCG GTG CGG ATG ACC TTC CAC 96
20 25	Ala Val Arg Met Thr Phe His
20 25	30
CTC CCC GGA GAC GCG CCT GTG ACC TGG	GCG TTC GGC CGG GAG CTG CTG 144
Leu Pro Gly Asp Ala Pro Val Thr Trp	Ala Phe Gly Arg Cly Low Low
35 40	A5
	and the second s
CTC GAC GGG ATC AAC CGC CCG AGC GGC	GAC GGC GAC GTC CAC ATC GCC 192
eu Asp Gly Ile Asn Arg Pro Ser Gly	Asp Gly Asp Val His Ile Ala
50 (25) (10) (25) (35) (55)	
• 1°	
CCG ACC GAC CCC GAG GGC CTG TCG GAC	GTC TCC ATC CGG CTC CAG GTG 240
oro Thr Asp Pro Glu Gly Leu Ser Asp	Val Ser Ile Arg Leu Gln Val
65 70	75 - 80

															GCC Ala	•	288
						AAG Lys										•	336
						AGC Ser				Ala	Leu	Gly 125		Ile	Leu	* 2	384
						GGC Gly 135				n Simon		erites Possibilit		7°. 13 è	\$1.5 2.5 4.7		408
(2)	INFO	ORMAT	rion	FOR	SEQ	ID N	10: E		f .1. ·	a					n unu. Ne en		
	((<i>I</i>	A) LI 3) T	ENGTH	I: 13 amir	RACTE 35 am 10 ac line	ino cid	acio			·. · . ·	a .	.7	3-47 417			
	(ii)	MOI	жстп	ne my	DR.	prot	ein									* *.	
	(xi)					PTIC			D · NO			:	\d\.				
Met 1		SEÇ	QUENC	CE DE	ESCRI	-)N: #8	EQ†] Leu	Ser 10): 8: Phe	Lys	Ile	AM AM •{ •	Val			
1	Ser	SEC Phe Tyr	Leu Glu 20	CE DE Val 5	Ser Ser Arg	PTIC	ON: *S Glu Pro	Leu Tyr 25	Ser 10 Ala	Phe Val	Lys Arg	Ile Met	Pro Thr	Val 15 Phe	Glu His		
1 Leu Leu	Ser Arg Pro	SEC Phe Tyr Gly 35	Leu Glu 20 Asp	Val 5 Thr	Ser Arg	Glu Asp Asp Asp Asy Val	ON: *S Glu Pro Thr 40	Leu Tyr 25	Ser 10 Ala Ala	Phe Val	Lys Arg	Ile Met Arg 45	Pro Thr 30	Val 15 Phe Leu	Glu Bis Leu	. ••	
1 Leu Leu	Ser Arg Pro	SEC Phe Tyr Gly 35	Leu Glu 20 Asp	Val 5 Thr	Ser Arg	Glu Asp Asp Asp Asy Val	ON: *S Glu Pro Thr 40	Leu Tyr 25	Ser 10 Ala Ala	Phe Val	Lys Arg	Ile Met Arg 45	Pro Thr 30	Val 15 Phe Leu	Glu His Leu	, 5,1 , 5,1	
Leu Leu Leu Pro 65	Arg Pro Asp 50	SECONDARY SECOND	Glu Glu 20 Asp Ile	Val 5 Thr Ala Asn	Ser Arg Pro Arg Gly 70	Asp Asp Asp Val A Lea Prose 55	Pro Thr 40 Ser	Leu Tyr 25 Trp Gly	Ser 10 Ala Ala Asp	Phe Val Phe Gly Ser 75	Lys Arg Gly Asp 60	Ile Met Arg 45 Val	Pro Thr 30 Glu His	Val 15 Phe Leu Ile	Glu His Leu Ala		
1 Leu Leu Pro 65 Gly	Arg Pro Asp 50 Thr	SECONDARY SECOND	Leu Glu 20 Asp Ile Pro	Val 5 Thr Ala Asn Glu Ala 85	Ser Arg Pro Arg Gly 70 Leu	Asp Val A Lea	ON: SET	Leu Tyr 25 Trp Gly Asp	Ser 10 Ala Ala Asp Val Gly 90	Phe Val Phe Gly Ser 75	Lys Arg Gly Asp 60 Ile	Ile Met Arg 45 Val Arg	Pro Thr 30 Glu His Leu	Val 15 Phe Leu Ile Gln Val	Glu His Leu Ala Val 80 Ala		
1 Leu Leu Pro 65 Gly	Arg Pro Asp 50 Thr Ala	SECONDARIAN SECOND	CUENC Leu Glu 20 Asp Ile Pro Arg 100	Val 5 Thr Ala Asn Glu Ala 85	Ser Arg Pro Arg Gly 70 Leu Asp	Asp Asp Val A Low Prose 55	ON: SGlu Pro Thr 40 Ser Ser Arg	Leu Tyr 25 Trp Gly Asp Ala Val 105	Ser 10 Ala Ala Asp Val Gly 90	Phe Val Phe Gly Ser 75 Ala	Lys Arg Gly Asp 60 Ile Pro	Ile Met Arg 45 Val Arg	Pro Thr 30 Glu His Leu Glu 110	Val 15 Phe Leu Ile Gln Val 95	Glu His Leu Ala Val 80 Ala		

Ala Glu Glu Gln Asn Ala Gly 130 135	en de la companya de La companya de la co
(2) INFORMATION FOR SEQ ID NO: 9:	ALCHEN STATE OF THE SECOND
(i) SEQUENCE CHARACTERISTICS (A) LENGTH: 34 base pair (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	rs 1 (4.4 C. p.) - Francis (4.4 c.)
(ii) MOLECULE TYPE: DNA (synt)	netic)
<pre>(vi) ORIGINAL SOURCE: (C) INDIVIDUAL ISOLATE:</pre>	ssgl fragment and state the state of the sta
(xi) SEQUENCE DESCRIPTION: SEQ	Q ID NO: 9: 47 H / 12 1-12 10 (2%)
GGCGAATTCG AACAGCTACG TGGCGAAGTC (The public and the second of the public second
(2) INFORMATION FOR SEQ ID NO: 10:	n de la companya di kacamatan kendalah di kecamatan di kecamatan di kecamatan di kecamatan di kecamatan di kec Bermula
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 32 base pair (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	general de la companya de la company
(ii) MOLECULE TYPE: DNA (synth	metic)
(vi) ORIGINAL SOURCE: (C) INDIVIDUAL ISOLATE:	ssg2
(xi) SEQUENCE DESCRIPTION: SEQ	ID NO: 10:
GTGGGATCCG TGCTCGCGGC GCTGGTCGTC T	°C 32
(2) INFORMATION FOR SEQ ID NO: 11:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 32 base pair (B) TYPE: nucleic acid (C) STRANDEDNESS: single	·

(ii) MOLECULE TYPE: DNA (synthetic)

(vi) ORIGINAL SOURCE: (C) INDIVIDUAL ISOLATE: ssg3 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11: GGGAATTCCA TATGCGCGAG TCGGTTCAAG CA THOUGHT A SECURE OF THE SECOND e et sal lie et d'ét. (2) INFORMATION FOR SEQ ID NO: 12: Change of the State of (i) SEQUENCE CHARACTERISTICS: (B) TYPE: nucleic acid (C) STRANDEDNESS: single to the state of the (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (synthetic) AND THE MARKET IN (C) INDIVIDUAL ISOLATE: ssg4 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12: CCGGTCAGCC GGCGTTCTGC TCCTC 25 Figure 5 continued eran in the control of the state of the stat an Turkera — Roman Alberta (1965) Burgaran (1965) and the state of t 57.5 was a second of the second of the second and particles are recognitive to

.